FOOD ANALYSIS

Typical Methods
and the Interpretation of Results

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FOOD ANALYSIS

PREFACE TO THE FOURTH EDITION

In the present edition the scope of the book remains practically as originally planned, and, although considerable new material has been added, careful elision of some that was obsolete or less necessary has kept the volume from becoming appreciably larger. The methods of analysis have been thoroughly revised and brought up to date. This is especially true in the chapters relating to alcoholic beverages, where considerable new material relating to the detection of postprohibition practices has been added. In the case of sugars in certain food products a revised and more complicated procedure has been necessitated by the fact that dextrose is now placed on a par with cane sugar. The section on colors, although these are found in far less variety than formerly, has been retained for its educational value, as stated in earlier editions, and new permitted dyes, including oil-soluble colors, have been added. Preservatives, on account of their less importance than in the past, have been condensed to a minimum.

To avoid possible misunderstanding, it might be worth while to emphasize that this is purposely not a book teaching "food chemistry," not a book on dietetics, and no attempt has been made to follow the fate of the foodstuffs in the body. It is primarily and simply a brief treatise on a particular branch of analytical chemistry, in which the analytical results must be definitely interpreted to determine the true character of the material examined. Further, since it is written chiefly for students and beginners, some of the more complicated or difficult procedures, used successfully by experienced analysts, are purposely given only by reference.

Acknowledgment is gratefully made by the author to friends and users of the book who have made numerous acceptable suggestions for the improvement of the new edition, and especially is he indebted to his friend and colleague, Dr. T. R. P. Gibb, for advice and assistance in the revision of the first chapter.

CAMBRIDGE, MASS.,   A. G. WOODMAN.
    May, 1941.

PREFACE TO THE FIRST EDITION

This book has grown out of the courses given to the author's students in food analysis during the last few years. Experience with these classes, which have used as textbooks mainly Leach's "Food Analysis" and *Bulletin* 107 of the Bureau of Chemistry, has shown the need of a book which should cover distinctly less ground than either of these, but should give to the student a more detailed discussion of the analytical processes involved, their suitability and limitations. Further, an attempt has been made to lay greater emphasis on the interpretation of the analytical results. To the author's mind the principal asset to be gained by the student from any detailed consideration of the methods employed to detect adulteration in foods is the exercise of judgment and the training of the sense of discrimination, which is derived from a critical balancing of the data obtained in a food analysis. Substances are being examined which are usually capable of wide natural variations in composition, and an exceptional opportunity is afforded for a critical study of the analytical factors in order to determine whether or not they imply artificial manipulation of the product.

Because the primary intention has been to write a book of the character outlined no effort has been made to include a great variety of food materials, nor necessarily those of greatest economic importance or which are most widely used. Certain typical foods have been selected to illustrate important methods of attack or characteristic methods of food analysis. In a word, the book has been written and the material selected primarily for the undergraduate student of analytical chemistry rather than for the practicing chemist.

The fact that certain typical foods have been selected should not be considered as implying any intention to limit the student to the particular examples cited. Other products, similar in general character to those discussed, involving the same or different forms of adulteration, will readily suggest themselves. These may be purchased, or sometimes more conveniently be prepared

in the laboratory, and given to the student for analysis and intelligent interpretation.

Three of the most important groups of foods, fats and oils, carbohydrate foods, and alcoholic beverages, have been treated at some length, general methods common to the group being taken up first in each case, followed by a more detailed discussion of several typical examples. The detection and identification of artificial colors has been treated perhaps more fully than is warranted by the actual importance of the subject because this part of the work frequently causes the student some difficulty, and adequate discussions of it are hard to find. Moreover, it affords excellent training in the detection of minute quantities of material through a systematic procedure.

The standard texts on food analysis and related subjects have been freely consulted, especially those of Leach, Browne and Sherman, as well as Allen's "Commercial Organic Analysis," and much valuable material selected. Particularly helpful have been found the publications of the Bureau of Chemistry, especially the bulletins comprising the annual proceedings of the Association of Official Agricultural Chemists, these furnishing access to the best of present American work in food analysis. For these reasons the methods chosen follow most closely typical American practice, although an earnest endeavor has been made not to slight the work of European food chemists.

Acknowledgment is gratefully made to the author's many friends who have aided by encouragement and advice, and especially to Mr. G. W. Rolfe and Mr. A. L. Sullivan, who have read the chapters on Carbohydrate Foods and Alcoholic Beverages, respectively, and made many valuable suggestions.

A. G. WOODMAN.

BOSTON, MASS.,
1915.

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FOOD ANALYSIS

CHAPTER I

GENERAL METHODS

Preparation of the Sample.—The material will often be in proper condition for analysis as received. The main points to be considered are whether the portion to be examined is of uniform composition and whether it is finely divided. Usually the first condition may be brought about by thorough mixing and sampling of the material; if large enough this may be done by the customary method of quartering. For the latter it may be ground in an ordinary food chopper or pulverized in a coffee or drug mill; or if small the sample may be ground in an ordinary porcelain mortar. In any case time will be well spent in making certain that the sample used for analysis is a truly representative one.

Specific Gravity.—In food analysis this determination has reference almost invariably to liquids. If only a fair degree of accuracy is necessary and sufficient sample is available, the determination can be made conveniently and quickly by the use of a hydrometer or specific-gravity spindle. An example is given in detail under Milk, page 127. If the amount of liquid is small, the specific gravity can be determined
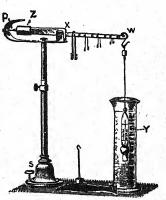
FIG. 1.—Westphal balance.

with somewhat greater accuracy by means of the Westphal balance. In principle this device is based upon the well-known law of physics that a body immersed in a liquid is buoyed up by a force equal to the weight of liquid displaced. The apparatus, shown in Fig. 1, consists of a beam balanced on a knife edge X and having a plummet or sinker Y suspended from one end and

counterpoised by a fixed brass weight Z at the other. The distance between X and the point of support W is divided by notches into 10 equal parts. The sinker, which is made of glass and provided with a thermometer, is made of such a size that it will displace exactly 5 grams of water at a standard temperature, usually 15.5°C. The plummet, the fine wire, and the hook by which it is suspended are made of a definite weight, usually 15 grams, so that they are interchangeable in different instruments.

In using the balance it is first adjusted by means of the leveling screw S until the pointer P on the arm is exactly opposite the reference point with the dry plummet hanging from the hook. If now the plummet be entirely immersed in distilled water at the standard temperature, it will require a weight of 5 grams at W to make it balance again. For liquids heavier than water a greater weight will be needed and for liquids lighter than water

0.9652   1.2260
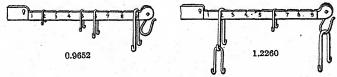
Fɪɢ. 2.—Reading the Westphal balance.

one correspondingly less. For reading the decimals of a unit of specific gravity, use is made of the notched divisions on the beam. Thus if the 5-gram weight placed directly over the plummet shows a specific gravity of 1.0000, the same weight placed 0.3 of the distance on the beam would correspond to a gravity of 0.3000. The second decimal is obtained by means of a 0.5-gram weight, the third by a 0.05-gram weight and the fourth by a 0.005-gram weight. Typical examples of the method of reading the instrument are shown in Fig. 2.

For more exact determinations of specific gravity some form of pyknometer should be used. Reduced to its lowest terms this apparatus consists of a light container for weighing equal volumes of liquids measured accurately at a definite temperature. A critical study of the sources of error in the use of the pyknometer, including precise determinations of specific gravity by a special form using a counterweight, has been made by Siebel and Kott.[1] Two common forms are the Sprengel-Ostwald tube (Fig. 3) and the specific-gravity bottle (Fig. 4).

[1] *J. Assoc. Off. Agr. Chem.*, **1937**, 535.

The Sprengel Tube.—This is especially useful when only a small amount of liquid is available for the determination or when the determination is to be made at a temperature quite different from room temperature. The best form is the one suggested by Ostwald and shown in the figure, one arm being a capillary tube while the other holds the bulk of the liquid. In using it the end B should be dipped into the liquid, which has been cooled to several degrees below the desired temperature, and the tube filled by applying suction through a rubber tube attached to the tip A. The pyknometer is then suspended in a narrow beaker filled with water at the desired temperature; and, when its contents have attained this temperature, as shown by the meniscus in the capillary remaining stationary, by touching the tip A with the edge of a filter paper the meniscus can be brought to the reference mark C. The pyknometer is then carefully wiped dry, suspended from the hook over the balance pan and
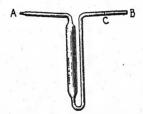
Fig. 3.—Sprengel-Ostwald tube.
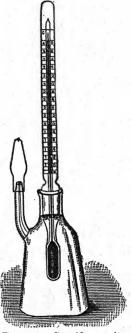
Fig. 4.—Specific-gravity bottle—Geissler form.

weighed. It is necessary also to calibrate the pyknometer, which is done by weighing it empty and dry, then weighing it filled with recently boiled distilled water in the manner just described.

The Specific-gravity Bottle.—These can be procured of various sizes, most conveniently of 25 or 50 cc. capacity. A common form is provided with a thermometer stopper as shown, extending to 40°C. One should be chosen with as large a cap to the side tube as possible, and it is best to have a very small hole drilled or blown in the extremity of the cap so that the liquid may expand

into the cap during the weighing without forcing it up and causing leakage. The thermometers are not always accurate and for careful work should be tested by comparison with one of known accuracy, or the bottle should always be adjusted by immersion in a bath of known temperature.

Calibration of the Pyknometer.—This is done by weighing the pyknometer empty and dry and then filling it with recently boiled distilled water at a temperature one or two degrees below that at which the determination is to be made. The pyknometer should be filled nearly full with the water so that when the thermometer is inserted the water will overflow through the capillary. The bottle is then placed in a bath of water kept at the desired temperature or allowed to stand near the balance (do *not* hold it in the hand to warm it more quickly); and as soon as its contents have reached that temperature, as shown by the thermometer, the excess of water is carefully wiped from the tip of the capillary, the cap placed on, and the bottle wiped dry and weighed. It should be obvious that it is the temperature at which the adjustment is made that is important. The temperature of weighing, if not too different, is immaterial. Subtracting the weight of the pyknometer gives the water content of the bottle at the temperature used, which is most conveniently 20°C., although 15.6°C. is sometimes employed. The determination should be repeated and the mean of two closely agreeing values recorded for use.

To determine the specific gravity of a liquid, the pyknometer thus calibrated is rinsed several times with the liquid with which it is to be filled, or it may be rinsed with alcohol and then with ether and dried in an oven at 100°C. (If this is done care should be taken that the thermometer is not put into the oven also, since this, registering to only 40°, will be broken.) The pyknometer is then filled with the liquid, using the same precautions as regards temperature as with distilled water, and weighed. The weight of the liquid contained, divided by the water content, is the specific gravity.

In stating the specific gravity of a liquid it is advisable to record the temperature at which the determination was made, as well as the temperature of the water with which it is compared. This is done in the form of a fraction thus, $\dfrac{20°}{20°}$, meaning the specific gravity at 20°C. referred to water at 20°C.

In order to determine the specific gravity at a given temperature $t°$, referred to water at its maximum density 4°C., sometimes called the *true* specific gravity or *density*, the value determined at $\frac{t°}{t°}$ should be multiplied by the density of water at $t°$, taken from Table 1, page 6.

Thus the value for $\frac{20°}{20°}$ must be multiplied by 0.998234 to obtain the value at $\frac{20°}{4°}$.

Index of Refraction.—When a beam of light passes obliquely from one medium to another of different optical density it is bent out of its course or *refracted*. For two given media the amount
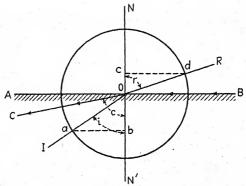
FIG. 5.—Illustrating the law of refraction.

of this refraction is a constant for any definite temperature and can be stated mathematically by the expression $\frac{\sin i}{\sin r} = n$, where i is the *angle of incidence* made by the incident ray with the perpendicular to the dividing surface and r is the *angle of refraction* made by the refracted ray with the perpendicular; n is called the index of refraction. For example, in Fig. 5 let AB be the surface of separation between two media, of which the upper is the rarer, and let a beam of light pass through in the direction RO. The angle RON, which the incident ray makes with the perpendicular, is the angle of incidence; and the angle ION' made by the refracted ray with the perpendicular is the angle of refraction. The index of refraction would be represented in the figure by the ratio $\frac{cd}{ab}$.

TABLE 1.—DENSITY OF PURE WATER FREE FROM AIR

(Under standard pressure, 76 cm., at every tenth part of a degree of the international hydrogen scale from 0 to 40°C., in grams per milliliter*)

Degrees Centigrade	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
0	0.999 868	874	881	887	893	899	905	910	916	921
1	926	931	936	940	945	949	953	957	961	964
2	967	971	974	976	979	982	984	986	988	990
3	992	993	995	996	997	998	998	999	999	†000
4	1.000 000	†999	†999	†999	†998	†997	†997	†996	†994	†993
5	0.999 991	990	988	986	984	981	979	976	974	971
6	968	965	961	958	954	950	946	942	938	934
7	929	924	920	915	910	904	899	893	888	882
8	876	870	864	857	851	844	837	830	823	816
9	809	801	794	786	778	770	762	753	745	736
10	728	719	710	701	692	682	672	663	653	643
11	633	622	612	602	591	580	569	558	547	536
12	524	513	501	489	478	466	453	441	429	416
13	404	391	378	365	352	339	325	312	298	285
14	271	257	243	228	214	200	185	171	156	141
15	126	111	096	080	065	049	034	018	002	986
16	0.998 970	954	937	921	904	888	871	854	837	820
17	802	785	768	750	732	715	697	679	661	642
18	624	605	587	568	549	530	511	492	473	454
19	434	415	395	375	355	335	315	295	275	254
20	234	213	193	172	151	130	109	087	066	044
21	023	001	†979	†958	†935	†913	†891	†869	†847	†824
22	0.997 801	779	756	733	710	687	664	640	617	593
23	570	546	522	498	474	450	426	402	377	353
24	328	303	279	254	229	204	178	153	128	102
25	077	051	025	†999	†973	†947	†921	†895	†868	†842
26	0.996 815	789	762	735	708	681	654	627	600	572
27	545	517	489	462	434	406	378	350	321	293
28	265	236	208	179	150	121	092	063	034	005
29	0.995 976	946	917	887	857	828	798	768	738	708
30	678	647	617	586	556	525	495	464	433	402
31	371	340	308	277	246	214	183	151	119	088
32	056	024	†992	†959	†927	†895	†863	†830	†797	†765
33	0.994 732	699	666	633	600	567	534	501	467	434
34	400	367	333	299	265	231	197	163	129	095
35	061	026	†992	†957	†923	†888	†853	†818	†783	†748
36	0.993 713	678	643	607	572	536	501	465	430	394
37	358	322	286	250	214	178	141	105	069	032
38	0.992 996	959	922	885	849	812	775	738	700	663
39	626	589	551	514	476	438	401	363	325	287
40	249	211	173	135	097	058	020	†981	†943	†904

* According to P. Chappuis, Bureau international des Poids et Mésures, *Travaux et Mémoires*, XIII. **1907**.

Ordinarily the index of refraction of a substance is taken as the ratio of the angles formed when light passes from *air* to the substance and is referred to the *D* ray of the spectrum as the standard wave length of light, so that for a temperature of 20°C. *n* would be written n_D^{20}.

The Critical Angle and Total Reflection.—Referring again to Fig. 5 it will be seen that if the light pass in the opposite direction, from the denser to the rarer medium, the angle of refraction *RON* will be greater than the angle of incidence *ION'*. If the angle of incidence be increased, then, at a certain angle of incidence, less than 90°, the angle of refraction will become 90°, *i.e.*, the refracted ray will coincide with the dividing surface. For incident rays striking the surface at a greater angle than this, the beam of light will be *totally reflected* and there will be no refracted ray. The angle of incidence at which this occurs is known as the *critical angle*.

Then since

$$n = \frac{\sin i}{\sin r},$$

at the critical angle

$$n = \frac{\sin i}{\sin 90°} = \frac{\sin i}{1} = \sin i.$$

That is, in passing from a denser to a rarer medium, the index of refraction is equal to the sine of the angle of incidence for the border line of total reflection.

The expression given is true of course only when light is passing into air. If air is not one of the media, but the light is passing, say from glass into some liquid, then the expression for the liquid would be $n = n_g \cdot \sin i$, where n_g is the refractive index of the glass referred to air.

Conversely, if the light is passing from the rarer to the denser medium in the direction *BO* (Fig. 5), so-called "grazing incidence," the angle of incidence is 90° and the beam is refracted at the critical angle *c*.

The forms of refractometer most commonly employed in food analysis are based upon this principle of measuring the critical angle for light striking the polished surface of a prism at grazing incidence.

Abbe Refractometer.—In this instrument the refractive index of a liquid is determined by measuring the critical angle for light passing from it into a glass prism of higher refractive index. The sine of this angle is the index of refraction of the liquid referred to glass and this, multiplied by the refractive index of the glass, as indicated above, gives the index of refraction of the liquid, referred to air.
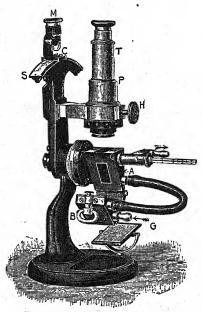
Fig. 6.—The Abbe refractometer.

The apparatus (Fig. 6) consists essentially of three parts:

1. Two prisms A and B of flint glass, having a refractive index of about 1.75, mounted so that they can be separated and a few drops of the liquid to be examined placed between, forming a thin layer when the prisms are joined again. The prisms can be rotated by means of a movable arm or alidade C which carries the reading magnifier M.

2. A telescope T provided with cross hairs by which the position of the border line of total reflection can be observed.

3. The sector S divided proportionally to the sines of the various angles of incidence for the border line of total reflection,

multiplied by the refractive index of the prisms, and therefore representing indexes of refraction.

An important part of the apparatus is the compensator, placed in the tube of the telescope at P and composed of two similar Amici prisms which can be rotated simultaneously in opposite directions by the milled head H, thus making available a range of dispersion varying from zero to twice the dispersion of a single prism in what may roughly be described as opposite directions. In this way the compensator can be given an equal but opposite dispersion to that of the liquid under examination and the border line of total reflection, which may appear as a colored band, owing to the fact that the critical angle varies with the wave length of light, can be changed to a sharp colorless line even when white light is used as the illuminant. It should be noted that the Amici prisms, composed of crown and flint glass, are so constructed that the yellow (D) ray of entering white light is transmitted without deviation, while rays of other wave lengths are refracted. Hence, while the dispersion due to the liquid is compensated, the reading for the refractive index, ordinarily stated in terms of the D ray, is unchanged.

To use the refractometer, the prisms and telescope are rotated as far as possible in the direction from the observer, the latch is turned to the right and the prism B swung open. Two or three drops of the liquid to be examined are then placed on the polished surface of the stationary prism A, great care being taken that nothing but the liquid is allowed to touch the surface of the prism, since it is easily scratched, and prism B is brought back into position and clamped as before. The whole instrument is then rotated toward the observer and the mirror G turned so as to reflect the light upon the prism B.

By means of the movable arm C the prisms are rotated in the field of the telescope until the border line of total reflection is observed, the lower portion of the field being dark and the upper portion bright. If the border line should be colored, this is corrected by means of the compensator, the eyepiece of the telescope is focused sharply, and by cautious movement of the arm, using the fine adjustment if one is provided on the instrument, the line is made to coincide with the junction of the cross hairs. The reading of the scale through the magnifier M gives directly the refractive index to the third and, by estimation, to

the fourth decimal. The temperature should be read by the thermometer to the nearest $\frac{1}{2}°$ before reading the index. After several settings and readings of the instrument have been made the prisms should be cleaned with alcohol and a clean soft cloth or with "Kleenex" tissue.

Temperature Regulation.—The refractive index of liquids is a function of the temperature, increasing as the temperature
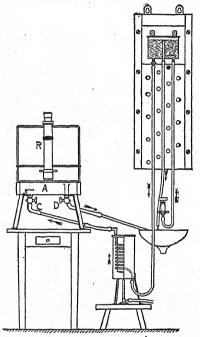
Fig. 7.—Refractometer heater.

decreases. It is hence necessary to note the temperature at which the reading is made. Moreover, with some substances, as the solid fats, it is essential that the temperature of observation should be above their melting points, so that some method of controlling and raising the temperature is desirable. This is done in the Abbe instrument by a current of water of the desired temperature which flows through the prism casing in the direction of the arrows shown in Fig. 6. This water may be supplied from a large reservoir of warm water, or the spiral heater supplied by the makers may be employed, as shown in Fig. 7, connected with the

immersion refractometer. The regulation of the temperature is accomplished in two ways, by varying the heat from the gas burner and by raising or lowering the upper reservoir on the slotted board.

A more recent method of temperature control is shown in Fig. 8. It consists of a constant-level column of water with a flow-regulating valve and a variable electric heater. The
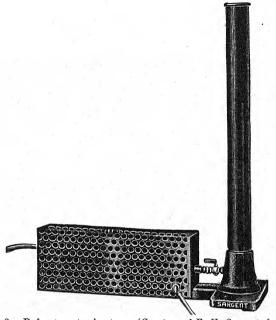
Fig. 8.—Refractometer heater. (*Courtesy of E. H. Sargent & Co.*)

temperature is adjustable from 5°C. above the input to 40°C. with an accuracy of ± 0.1°C. or better.

The temperature at which the determination was made should always be stated in reporting a reading.

The correctness of the adjustment of the instrument should be tested from time to time. This may be done for the lower portion of the scale by taking the average of several readings on distilled water, the refractive index of which for ordinary temperatures is shown in Table 2. The makers of the instrument furnish with it a glass test plate of known refractive index which may also be used. The plate, which has two polished surfaces, is

pressed onto the polished surface of the upper prism by a drop of liquid of high refractive index, commonly monobromnaphthalene, placed between the two, and light reflected from the lower prism mounting enters the prism at grazing incidence to form the edge of the shadow. Full details of the procedure may be found in the circular that comes with the instrument or in Browne, "Handbook of Sugar Analysis," page 59. In this case, dealing with a solid, the temperature may be neglected. The necessary adjustment may be made by moving the reticule with the small key supplied with the instrument; or, if the error is slight, it may be better simply to correct the reading. The Abbe instrument, if in adjustment, should give the refractive index to 0.0002.

TABLE 2.—REFRACTIVE INDEX OF WATER

Temperature, °C.	Refractive index	Temperature, °C.	Refractive index
18	1.3332	23	1.3327
19	1.3331	24	1.3326
20	1.3330	25	1.3325
21	1.3329	26	1.3324
22	1.3328	27	1.3323

Figure 9 illustrates diagrammatically the passage of light through the instrument, although the thickness of the liquid film and the deviations in path of the essential rays are necessarily greatly exaggerated. Light traveling through the lower prism reaches the finely ground hypotenuse surface, which becomes the point source for light that passes through the liquid layer, and, striking the polished surface of the upper prism at grazing incidence, is refracted at the critical angle into the telescope. Two only of these light points are shown in the figure.

Some of the rays, a, a', are totally reflected, the others transmitted, the critical angle for a given pair of prisms being dependent on the refractive index of the liquid. The rays b, b' coincide with the surface of the lower prism. The layer of liquid being very thin, ordinarily about 0.1 mm., the first rays, c, c', whose angle of incidence is infinitesimally less than 90°, strike the polished surface of the upper prism at practically grazing incidence and are transmitted to form the edge of the shadow.

Rays similar to d, d', emerging into the liquid at angles less than the critical angle, form the light portion of the field.

The Immersion Refractometer.—This instrument, of later construction than the Abbe refractometer, is capable of considerably greater delicacy. In its original form it is, on the other hand, more limited in its range, giving indexes of refraction between 1.32 and 1.36 only.
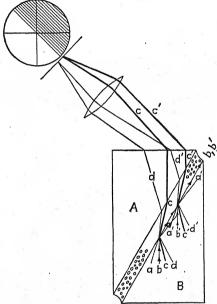
Fig. 9.—Diagram of Abbe refractometer.

A more recent form may be obtained with a series of interchangeable prisms, the range of 1.32 to 1.54, for example, being covered by a set of six prisms. They are readily removed from the instrument and replaced, the only disadvantage being that each prism when replaced must be carefully tested with a known liquid or test plate for the accuracy of its reading. For very small amounts of liquid an auxiliary prism can be used, the few drops of liquid being placed between the two as with the Abbe form.

The principle on which the immersion refractometer is based is the same as in the Abbe instrument, depending upon the

position of the border line for the critical angle of light refracted from grazing incidence.

Referring to Fig. 10, light is reflected from a mirror S so as to pass at grazing incidence, *i.e.*, nearly parallel to the oblique surface of the prism P. This prism, it will be observed, corresponds to the upper, or refracting prism, of the Abbe refractom-
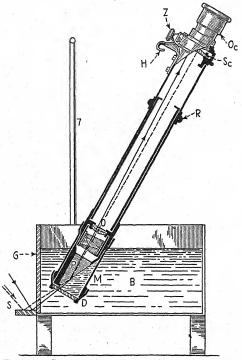
FIG. 10.—Construction of immersion refractometer.

eter. Rays that coincide with this surface form the border line of total reflection and are refracted upward through the prism at the critical angle in some such direction as the dotted line PO. Rays of light that strike the surface obliquely, as the solid line, are refracted and illuminate the upper portion of the field. Since there can be no greater angle of refraction in the prism than that for the border line of total reflection, the lower portion of the field remains dark, the location of the edge of the shadow varying with the refractive index of the liquid. The elimination

of undesirable rays is also aided by the ground cylindrical surface of the prism.

The construction of the refractometer is clear from the figure. *P* is the glass prism and at the other end of the tube is the ocular *Oc*. At *Sc* is the arbitrary scale of 110 divisions on which is read the degree of refraction. At *A* is a compensator of the same nature as that in the Abbe instrument and serving the same purpose. Only one Amici prism of high dispersion is used,
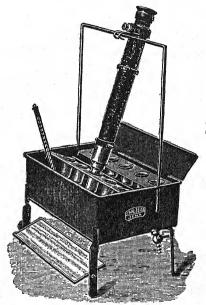
Fig. 11.—Method of using immersion refractometer.

however, strong enough to compensate most liquids. If not, the reading can be made in sodium light. This prism is connected by an inside sleeve with the milled ring *R* by which it can be rotated. At *Z* is an ingenious vernier by which fractional parts of a degree may be read on a revolving drum that moves the lens on which the scale is engraved. Each division on the micrometer drum equals 0.1 scale division.

In using the refractometer the solution to be examined is contained in a small beaker kept in a bath at constant temperature, as shown in Fig. 11, or the solution is more conveniently placed in the small metal cup which may be attached directly to the

end of the instrument as M in Fig. 10. This is especially advantageous with volatile liquids. The temperature of the bath should be noted or kept constant to within 0.1°. This is important because the average temperature coefficient of liquids is about 0.00037. The refractometer is hung from the wire frame so that the light from a window or a lamp is reflected by the mirror upward through the ground-glass plate in the bottom of the bath and through the prism. Strong illumination and

TABLE 3.—REFRACTIVE INDEXES (n_D) CORRESPONDING TO READINGS OF THE IMMERSION REFRACTOMETER

Scale reading		Scale reading		Scale reading		Scale reading		Scale reading	
0	1.32736	20	1.33513	40	1.34275	60	1.35021	80	1.35750
1	1.32775	21	1.33551	41	1.34313	61	1.35058	81	1.35786
2	1.32814	22	1.33590	42	1.34350	62	1.35095	82	1.35822
3	1.32854	23	1.33628	43	1.34388	63	1.35132	83	1.35858
4	1.32893	24	1.33667	44	1.34426	64	1.35169	84	1.35894
5	1.32932	25	1.33705	45	1.34463	65	1.35205	85	1.35930
6	1.32971	26	1.33743	46	1.34500	66	1.35242	86	1.35966
7	1.33010	27	1.33781	47	1.34537	67	1.35279	87	1.36002
8	1.33049	28	1.33820	48	1.34575	68	1.35316	88	1.36038
9	1.33087	29	1.33858	49	1.34612	69	1.35352	89	1.36074
10	1.33126	30	1.33896	50	1.34650	70	1.35388	90	1.36109
11	1.33165	31	1.33934	51	1.34687	71	1.35425	91	1.36145
12	1.33204	32	1.33972	52	1.34724	72	1.35461	92	1.36181
13	1.33242	33	1.34010	53	1.34761	73	1.35497	93	1.36217
14	1.33281	34	1.34048	54	1.34798	74	1.35533	94	1.36252
15	1.33320	35	1.34086	55	1.34836	75	1.35569	95	1.36287
16	1.33358	36	1.34124	56	1.34873	76	1.35606	96	1.36323
17	1.33397	37	1.34162	57	1.34910	77	1.35642	97	1.36359
18	1.33435	38	1.34199	58	1.34947	78	1.35678	98	1.36394
19	1.33474	39	1.34237	59	1.34984	79	1.35714	99	1.36429
								100	1.36464

careful adjustment of the mirror will give the best results. The milled ring R is turned until the border line is free from color and the ocular Oc focused so that the line and the scale are both sharp. The upper portion of the field will be bright and the lower portion dark, the position of the border line upon the

graduated scale indicating the degree of refraction. If the line comes between two of the scale divisions its exact position may be read by the micrometer Z as follows: First, with the micrometer set at 0 read the next lower whole division on the scale, then turn the micrometer screw until the line coincides exactly with a scale division. From the graduated drum of the micrometer the exact reading may be made to hundredths of a division. The reading should be the average of several settings and should not be taken until the instrument has been kept for at least 10 minutes at the temperature of the bath. Much trouble may be avoided and a sharp line obtained instead of a hazy one by being sure that the temperature has become constant. The reading may be reported directly in scale divisions or may be converted to the corresponding refractive index by Table 3, page 16.

In using this conversion table it should be noted that it applies only to the Zeiss immersion refractometer, the arbitrary scale of which is based on that suggested by Pulfrich[1] in which 14.5 corresponds to a refractive index of 1.33300, 50 to 1.34650 and 100 to 1.36464. Some of the instruments made by Bausch and Lomb (Serial Nos. 4,000 to 10,000) have a somewhat different scale, the difference in values amounting to practically 1 per cent at the 100 mark for the low-range prism commonly used. With the prisms of higher range the differences are much greater. The later Bausch and Lomb instruments have the Pulfrich scale. A table enabling the use of Bausch and Lomb refractometers with the modified scale (Serial Nos. 4,000 to 10,000) in comparison with the Zeiss scale, taken from the report of the committee of the Association of Official Agricultural Chemists,[2] is given on page 18.

Adjusting the Refractometer.—This may be done conveniently by taking a reading on distilled water, which should read 14.5 on the scale at 20°C. The same precaution should be observed as described above in regard to attaining the temperature, and R should be set at 5 for the Zeiss instrument or 2 for the Bausch and Lomb. It will perhaps be better to have the reading on water precede the actual determination both for the practice in using the instrument and to ascertain if it is correctly adjusted.

[1] *Z. angew. Chem.*, **1899**, 1168.
[2] *J. Assoc. Off. Agr. Chem.*, **1933**, 87.

If a slight error in reading is found, the instrument may be readjusted by a change in the micrometer screw. Directions for doing this will be found in Browne, "Handbook of Sugar Analysis" or in Leach-Winton, "Food Inspection and Analysis," but it will be advisable for the student to make the necessary corrections rather than attempt this. In every case, after being used, the prism should be carefully wiped dry with a soft clean cloth or "Kleenex" tissue, and, since the instrument is necessarily moved about, the unprotected prism must be handled *with the greatest possible care.*

Scale reading, B & L modified serial nos. 4,000 to 10,000	Refractive index	Scale reading, Zeiss
−5	1.32539	−5.0
0	1.32737	0.0
+5	1.32934	+5.1
10	1.33131	10.1
15	1.33326	15.2
20	1.33521	20.2
25	1.33714	25.2
30	1.33907	30.3
35	1.34098	35.3
40	1.34289	40.4
45	1.34478	45.4
50	1.34667	50.5
55	1.34855	55.5
60	1.35041	60.5
65	1.35227	65.6
70	1.35411	70.6
75	1.35595	75.7
80	1.35778	80.8
85	1.35959	85.8
90	1.36139	90.8
95	1.36319	95.9
100	1.36497	100.9
105	1.36674	106.0

Determination of Moisture.—The method most commonly employed for the determination of moisture in foods is by drying at the temperature of boiling water.

A convenient weight, usually 2 to 10 grams, is spread in a thin layer in a flat-bottomed dish or on a watch glass and dried in an

oven surrounded by boiling water until the weight, at intervals of
½ hour, remains constant. The loss in weight is taken as water.

A convenient copper oven which serves both for drying and for
evaporating is shown in Fig. 12, dishes of various sizes being
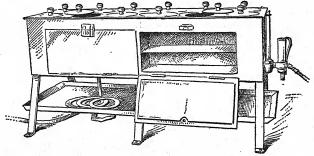
Fig. 12.—Oven and water bath.

placed on copper rings over the openings and the drying being
done on shelves within the oven. A constant-level tube may be
provided at one end to be connected with the water supply.
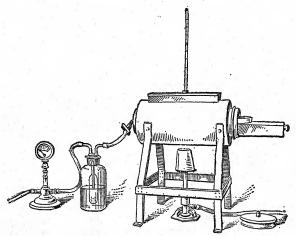
Fig. 13.—Vacuum drying oven.

This particular bath is heated by lamps beneath, although a
steam coil within the bath itself can be used to advantage
and should be when evaporating inflammable liquids like ether.
Electrically heated ovens are more commonly used if the temper-

ature is to be varied from that of boiling water.   They are relatively inexpensive, and the temperature is automatically regulated.   Usually a period of from 2 to 5 hours is sufficient to dry the sample.   There are several precautions to be observed, and the exact details of time, temperature, etc., depend somewhat upon the character of the material to be dried.

With some substances water is expelled rather slowly at the temperature of boiling water, which rarely reaches 100°C., and in such cases an air oven heated to 105°C. is preferable.   On
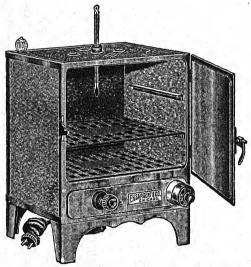
FIG. 14.—Electric drying oven.   (*Courtesy of E. H. Sargent & Co.*)

the other hand, in some cases the temperature of boiling water is too high for correct results.   Levulose is decomposed in the presence of water at temperatures in excess of 70°C. so that the method cannot be used for such products as honey, jams, and fruit juices, which contain this sugar in appreciable amounts.   Such materials should be dried at 70°C. *in vacuo*, or, since many laboratories are not equipped with the necessary apparatus for this, densimetric or refractometric methods are commonly employed. These, being generally used for carbohydrate foods, are discussed in Chap. VI.   A simple form of copper oven as used in the author's laboratory for drying in a partial vacuum or in a current of inert gas is shown in Fig. 13.

A still simpler form of vacuum oven, which is suitable for small amounts of substance and can be made from laboratory materials, is described by Browne.[1]

Further, if the food materials contained substances other than moisture that are volatile at 100°C., they would be included in the moisture determination made in the manner indicated. Such a case would be the volatile oil in spices. The determination can be made in such cases by drying the material without heat, best *in vacuo* over concentrated sulphuric acid, but the method is slow and tedious, requiring several days for its completion.

If it is desired to dry liquid foods that are thick or sirupy they should be spread upon some absorbent material in the dish, as sand, asbestos, or pumice.

Direct Determination of Water.—Some of the disadvantages of drying may be avoided, with a gain in time and accuracy, if the moisture be determined by distillation of the sample with a liquid immiscible with water.[2] The distillate is received in a calibrated sedimentation tube with a side arm, which returns the medium to the distilling flask while the water is trapped in the calibrated tube. The method is applicable to a wide variety of food products, even in the presence of levulose, by using toluene for the distilling liquid, can be carried out in about 1 hour, and does not necessitate complicated or expensive apparatus.

The apparatus recommended by Bidwell and Sterling is shown in Fig. 15, the essential dimensions being given. The calibrated receiving tube is conveniently made from a graduated section from a 5-cc. Mohr pipette, sealed at the lower end and attached to the funnel tube.
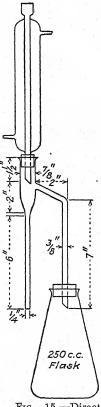
FIG. 15.—Direct determination of water.

250 c.c. Flask

[1] "Handbook of Sugar Analysis," p. 23.

[2] MARCUSSON: *Mitt. k. Materialprüfungsamt,* **1905,** 58; DEAN and STARK: *Ind. Eng. Chem.,* **1920,** 486; BIDWELL and STERLING: *J. Assoc. Off. Agr. Chem.,* **1925,** 295.

Procedure.—Introduce into the 250-cc. Pyrex Erlenmeyer flask enough sample to give from 2 to 5 cc. of water. If the sample, as in the case of flour, is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely, usually about 75 cc., and connect the apparatus as shown. Fill the receiving tube with toluene by pouring it through the top of the condenser. Bring to a boil and distill slowly, about 2 drops per second, until most of the water has passed over; then increase the rate of distillation to about 4 drops per second. When the water is apparently all over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distill over; if it does, repeat the washing-down process. If any water remains in the condenser, remove it by brushing down with a tube brush attached to a copper wire and saturated with toluene, washing down the condenser at the same time. The entire process should not take over 1 hour. Allow the receiving tube to come to room temperature. If any drops adhere to the sides of the tube they can be forced down by a rubber band wrapped around a copper wire. Read the volume of water to hundredths of a cubic centimeter and calculate to percentage.

Notes.—The distillation can usually be conducted with a free flame, although it is advisable to use a bath for samples containing sugar.

It is necessary to have the condenser and receiving tube chemically clean in order to prevent an undue quantity of water sticking to the condenser and drops of water adhering to the sides of the receiving tube. Clean with bichromate cleaning solution, rinse with alcohol, and dry in an oven. If the tube is cleaned before each determination and the condenser for every two or three determinations, it will not be necessary to brush down the condenser so much for the removal of drops of water.

Calderwood and Piechowski[1] use xylene and remove adhering droplets of water by the addition of two or three drops of ethyl alcohol. Figures are given to show the accuracy of the method.

Although the Bidwell and Sterling modification of the Dean and Stark apparatus has been made official by the Association

[1] *Ind. Eng. Chem., Anal. Ed.*, **1937**, 520.

of Official Agricultural Chemists for moisture in grain and stock feeds and is stocked by the dealers in chemical apparatus, others[1] have suggested notable improvements, among which are the use of Pyrex glass entirely, the replacement of corks by ground-glass joints, and the use of condensers in which the water and vapors travel downward. A form in which the condenser hangs tightly in the upper portion of the receiving tube, making it much easier to clean and to remove water droplets, is shown in Fig. 16.

Advantages of the method over oven drying are that it determines water directly, and not merely loss in weight. The effects of humidity during the drying and weighing and the danger of oxidation and skin formation while in the oven are eliminated.

Determination of Ash.—It is helpful with many foods to determine not only the total ash but the ash soluble and insoluble in water, the alkalinity of the soluble ash, and the proportion of ash insoluble in acids.

Total Ash.—This determination may be made on the sample used for the determination of moisture, or a fresh portion may be taken. In either case, the sample should be in a weighed platinum dish, preferably flat-bottomed, and should be ignited gently over a small flame until thoroughly charred. The dish is then placed in

F I G . 1 6 .— Calco apparatus for direct determination of water. (*Courtesy of Eimer & Amend.*)

a muffle and heated to low redness until a white ash is obtained, when it is cooled in a desiccator and weighed. A small Pyrex beaker can be used for the determination of total ash but is not entirely satisfactory for accurate determinations of soluble ash and alkalinity of ash.

A small, inexpensive muffle furnace, capable of taking several small dishes, with a built-in rheostat and indicating pyrometer, is shown in Fig. 17.

If the total ash only is desired a small quantity (2 cc., pipetted) of an alcoholic solution of magnesium nitrate added helps in

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[1] TATE and WARREN: *Analyst,* **1936**, 367; DE LOUREIRO: *J. Assoc. Off. Agr. Chem.,* **1938**, 645; BECKEL, SHARP and MILNER: *Ind. Eng. Chem., Anal. Ed.,* **1939**, 425.

burning off the ash. Allowance may be made for the small amount of magnesium oxide left by means of a blank test.

The essential point to bear in mind in this determination is that the ash should not be heated to too high a temperature. There is danger that volatile material, as chlorides of the alkalies, will be driven off, or on the other hand, that a portion of the ash will be fused and enclose carbon which will thus escape ignition.
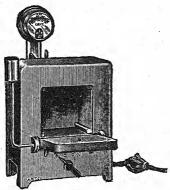
FIG. 17.—Electric muffle furnace. (*Courtesy of Arthur H. Thomas Co.*)

If the ignition is made in a muffle the temperature should be so regulated that it does not exceed 600 to 650°C., best determined by a thermoelectric couple placed directly in the muffle; if by direct heating of the dish, the latter should be nowhere heated to more than just visible redness. The ash determination, like the moisture, although apparently very simple, is in reality difficult to make satisfactorily. A good ash determination may require several hours, and the resultant ash should be of a uniform white or gray color, occasionally reddish or green, and free from fused lumps or particles of unburned carbon.

If it is difficult to secure a white ash in this manner the mass should be treated several times with hot water and filtered through an ashless filter. The filter and residue are ignited in the original dish, the filtrate added to the dish, evaporated to dryness on the water bath, and the whole ignited for a few moments at a low red heat.

Sirups or foods that contain much water must be heated carefully. Not over 5 to 10 grams should be taken and a dish of at least 50 cc. capacity is best. The dish should be heated cautiously with a low flame until the material begins to char; then by placing the flame at one side of the dish it is generally possible to regulate the charring so that it shall spread gradually over the dish without excessive foaming. A bit of vaseline placed on the mass is sometimes a considerable help and adds nothing to the ash.

The use of a muffle, although convenient, is not essential, for the ashing will usually proceed properly if the dish is heated directly with a small flame in a place free from draughts and if the material be stirred from time to time with a stout platinum wire. Covering the dish loosely with a sheet of platinum foil hastens the process by reflecting heat down upon the material.

In using platinum avoid ashing materials that may contain phosphates or even small amounts of lead or similar metals on account of possible danger to the dish.

Caution is also necessary in handling the dish containing the ash on account of the light, fluffy, skeleton ash often obtained, which is easily blown from the dish. While carrying the latter and even while it is in the desiccator, it is best covered with a small watch glass.

Soluble and Insoluble Ash.—To determine the *ash insoluble in water*, after weighing the total ash, add about 25 cc. of water to the dish, cover it with a watch glass to avoid loss by spattering, and heat it nearly to boiling. Filter through an ashless filter and wash with an equal volume of hot water. Place the filter paper and residue again in the dish, ignite and weigh. From the weight calculate the *water-insoluble ash* and by difference the *water-soluble ash.*

Alkalinity of Water-soluble Ash.—Some food products contain notable amounts of organic acids which during ignition become converted in part into carbon dioxide, held by the alkaline salts of the ash as carbonates, largely as potassium carbonate. A decreased quantity of alkali salts in the ash would hence be of some importance as indicating adulteration. On the other hand, an increase in the alkaline character of the ash may indicate chemical treatment, as in the case of cocoa. The determination of the alkalinity of the ash may therefore be of decided value. The determination is carried out in the following manner:

Allow the filtrate from the insoluble ash to cool and titrate with $0.1N$ hydrochloric acid, using methyl orange as an indicator. Report the result as the number of cubic centimeters of $0.1N$ acid required to neutralize the ash of 1 gram of sample.

Alkalinity of Insoluble Ash.—To determine this add 15 cc. of $0.1N$ hydrochloric acid to the weighed insoluble ash in the platinum dish, cover with a watch glass and heat cautiously nearly to boiling, over a small flame. Allow it to cool and titrate

the excess of acid with $0.1N$ sodium hydroxide, using methyl orange as before. The result is expressed as in the previous determination.

Ash Insoluble in Acid.—This determination is of value in showing added mineral matter, such as dirt or sand in spices, talc in confectionery, etc.   The weighed residue in the determination of ash insoluble in water may be used, or if this has been used in the determination of alkalinity, a fresh 2-gram portion of the original sample may be ignited and used directly.   To the ash in the dish, in either case, add 25 cc. of 10 per cent hydrochloric acid (sp. gr. 1.050), cover with a watch glass and boil gently over a low flame for 5 minutes, filter through an ashless filter, wash with hot water, return the filter and residue to the dish, ignite, and weigh.

Colorimetric Determinations.—For the determination of some constituents present in small amount, for instance citral in lemon extracts, vanillin in vanillas, or traces of lead in food products by means of the color given with diphenylthiocarbazone ("Dithizone"), methods depending upon the quantitative determination of color are frequently used.

Measurements of a fair degree of accuracy can be made by comparing the colors directly in tubes with smooth, polished bottoms, as the ordinary Nessler tubes used in water analysis; or two ordinary graduated cylinders can be selected which are of about the same diameter and have clear glass in the bottoms, free from distortion.   If one of these is used for the sample to be examined and the other contains a standard solution of nearly the same degree of color, then, if small portions of the deeper colored solution are removed until the colors are of equal intensity, the strength of the two solutions will be inversely proportional to the heights at which they stand in the cylinders.   It is always advisable to test the accuracy of the comparisons under the conditions of the experiment by dividing a colored solution into two exactly equal parts, placing them in the two cylinders and noting the readings.   It is also best to check the reading with the relative position of the cylinders reversed, since some observers invariably see the right- or left-hand tube deeper in color than the other.

It is important, and the same holds true of more elaborate colorimeters, that the standard and the unknown solution should

be reasonably close to the same intensity of color, since for some substances the depth of color is not exactly proportional to the strength of the solution for all concentrations.

The fundamental concept (Beer's law) may be stated: *The intensity of color of a solution is proportional to the concentration of the solute and to the depth of solution between the light source and the observer.* Hence

$$\log I_1 = kl_1c_1,$$
$$\log I_2 = kl_2c_2,$$

where I = observed intensity of color,
l = depth of solution,
c = concentration of given coloring agent, and
k = constant of proportionality.

When two intensities match, $I_1 = I_2$, and $\frac{c_1}{c_2} = \frac{l_2}{l_1}$. Beer's law assumes that the number of colored particles in a given solution does not change with the concentration. If the colored substance dissociates (or ionizes, rearranges, or combines with the solvent) to give products of a different hue from the original substance, and if the degree of dissociation, etc., varies with the concentration, Beer's law is obviously inapplicable. Where Beer's law does not apply, correction curves can be made using known solutions, or an empirical formula (Kober's) may be determined experimentally, as given under the Duboscq colorimeter.

A typical colorimeter and possibly the one in most common use is the Duboscq form shown in Fig. 18. The liquids to be compared are placed in the two cups, which have plane and polished bottoms. The cups can be raised or lowered so that the depth of liquid examined is in each case that between the bottom of the interior glass plunger and the bottom of the cup. This depth can be read to 0.1 mm. by a scale and vernier. Light is reflected through the solutions by a mirror, or a milk-white reflector can be substituted for the mirror if desired. The light is reflected by two prisms in the upper portion of the instrument, into the telescope, the field of which appears divided into two parts, permitting an exact comparison of the two colors.

The path of the rays of light in the instrument will be readily understood from the diagram in Fig. 19.

If the test does not have to be made with the greatest accuracy, a simple comparison of the colors of the sample and standard, previously adjusted by trial so that the final readings of the two are not far apart, will suffice.
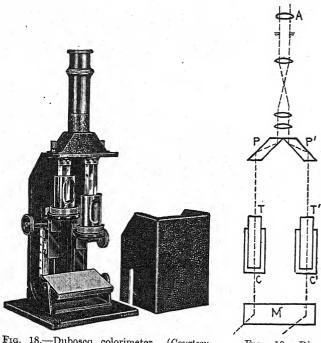
FIG. 18.—Duboscq colorimeter. (*Courtesy of Arthur H. Thomas Co.*)

FIG. 19.—Diagram of Duboscq colorimeter.

If a = the reading of the scale for the standard solution,

b = the reading of the scale for the unknown solution,

P = the percentage of substance in the standard solution, and

X = the percentage of substance in the unknown solution,

then

$$X = \frac{aP}{b}.$$

If, however, determinations of the highest accuracy are desired, corrections should be included in the course of the procedure for any possible deviations from Beer's law. In this case the detailed procedure would be as follows:

Method.—Rinse the cups with distilled water and then with the unknown solution to be tested. Add 15 cc. of the unknown solution to both cups and set them in place. Set the right-hand cup at 20 mm. and vary the height of the left-hand cup until illumination in both halves of the field is equal. Make 10 separate readings and set the left-hand cup at the average of these readings. Discard the solution in the right-hand cup. Rinse the cup and plunger with the standard solution and then add 15 cc. of this standard solution to the cup. Now adjust the position of the right-hand cup until the illuminations are equal. Take 10 readings. Average reading $= Y$.

Again discard the solution in the right-hand cup. Dilute the standard solution to exactly two-thirds of its previous strength, add 15 cc. of this diluted standard to the right-hand cup, and again adjust its position to obtain a match. Take 10 readings. Average $= Y'$. Discard the solutions. Rinse the cups and plungers several times with distilled water and dry with a towel.

From Kober's formula:

$$S = \frac{Y}{X} - \frac{(1 - X)YK}{X^2},$$

and also

$$S = \frac{Y'}{X'} - \frac{(1 - X')Y'K}{(X')^2},$$

where S = scale reading of unknown (in this case $S = 20$ mm.),
   Y = scale reading of standard,
   X = (concentration of unknown)/(concentration of standard), and
   K = constant expressing deviations from Beer's law.
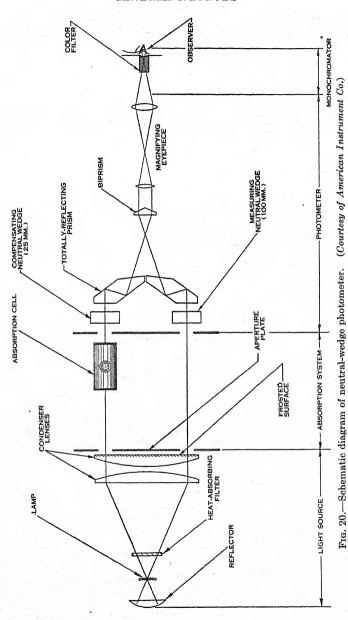
Substituting measured values of Y and Y', and noting that $X' = 1.5X$, solve for K. Use this value of K to find X and thus to find the concentration of the unknown. If Beer's law holds, $K = 0$. If K does not $= 0$ and if many determinations of the same kind are to be made, a calibration curve is usually plotted for the instrument.

Note that the setting of the left-hand cup does not enter into the calculations. It merely acts as a "tare." This procedure automatically corrects for any zero-point error of the instrument.

Photometers.—The type of colorimeter, of which the Duboscq is perhaps the most common example, measures in general the transmission value of a solution for the total spectral energy of the light source. But many substances absorb certain ranges of wave lengths of light from the normal spectrum, transmitting only the remainder. Accurate and much more delicate determinations of concentration of such substances may be made by measuring the intensity of the unabsorbed light at the wave length corresponding to maximum absorption for the substance. This can be done by means of a light filter, limiting the transmitted light to a spectral band, or better, by using a definite portion of the spectrum given by a prism or diffraction grating.

To explain more fully: A solution has a definite color primarily by reason of the wave lengths of the light absorbed, the remainder being transmitted. A red solution appears red because it absorbs all wave lengths but red. The absorption is often more selective than the transmission. For example, a solution may appear purple because it absorbs green. In making comparisons of the purple color in an ordinary colorimeter there will be several concentrations near together that cannot be distinguished because they all transmit purple light readily. The *real* problem, however, is to measure the absorption of the green light, which cannot be done easily by the eye because so much purple is transmitted. By using a filter that transmits only green light of the wave length absorbed by the solution, excluding the purple, the problem is simplified and the determination is more exact, because the real factor that varies with the concentration is the absorption of the green color. The measurement of the absorbed light may be made by visual comparison, or electrically by means of a photoelectric cell and a potentiometer or sensitive galvanometer.

Neutral-wedge Photometer.—An illustration of the first kind of apparatus, which is finding increasing use in food analysis, is the neutral-wedge photometer, shown in diagram in Fig. 20. In this a "neutral" wedge of dark glass, cemented to a similar wedge of optical glass to correct the refraction, is used as a standard of brightness. The absorption of light in the neutral wedge is considerable and practically uniform throughout the spectrum. The relative position of the wedge is read on a scale that is calibrated for a particular length of cell and a definite

FIG. 20.—Schematic diagram of neutral-wedge photometer. (*Courtesy of American Instrument Co.*)

monochromatic light filter by setting up a relationship between the scale reading and the known concentration of a solution in the cell. Once calibrated, the scale can be employed in subsequent determinations without the need of making up standards. Owing to the use of color filters, the matching, although visual, is one of brightness only and does not involve the matching of colors, which increases the degree of precision. The filter used should be selected to transmit the kind of light that is absorbed by the solution, as a red filter for a green solu-
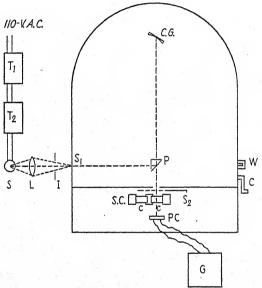
Fig. 21.—Diagram of spectrophotometer.

tion. The proper filter can be determined more exactly, if needed, from spectrophotometric measurements of the solutions being tested.

Spectrophotometer.—Comparisons may be made even better in some cases by using a more nearly monochromatic light source and measuring electrically the intensity of the light transmitted by the solution. From a uniformly controlled source of white light a spectrum is produced by a prism or diffraction grating. The prism or grating is so mounted on a calibrated rotating table that any desired portion of the spectrum may be selected. The narrow beam of monochromatic light passes through a cell

containing the solution being examined and is collected in a suitable linear-response photocell. The output of the photocell, which is in general proportional to the amount of light transmitted by the solution, especially if the incident radiant energy is small and is confined to a narrow spectral region, is measured by a sensitive potentiometer circuit or by a suitable galvanometer. By substituting a standard solution in the same light path, the ratio of light intensity at the selected wave length can be determined directly as per cent transmittance or as any desired quantitative function. A schematic diagram of such an arrangement is shown in Fig. 21.

In the diagram the letters have the following significance:

T_1 = constant-voltage transformer.
T_2 = 6-volt transformer.
S = 6-volt, 108-watt ribbon-filament bulb.
L = condensing lens.
I = iris diaphragm.
S_1 = adjustable entrance slit.
P = reflecting prism.
CG = concave grating.
S_2 = interchangeable exit slit (20, 10, and 5μ).
SC = sliding carriage.
c, c = absorption cells.
PC = barrier-layer photocell.
G = galvanometer.
C = crank which moves grating.
W = wave-length scale.

These forms of apparatus have proven of immense value, for example, in the determination of very small amounts of metallic impurities, notably lead, in food products. For this purpose the extremely delicate color reaction given by lead and several other metals with diphenylthiocarbazone, the so-called "dithizone" reaction,[1] has been commonly employed. Space does not permit of more than this brief mention. For details of theory and operation reference should be made to the books listed at the end of this chapter.

Centrifugal Methods.—An important piece of apparatus for the food laboratory is a suitable centrifuge. A comparatively simple

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[1] FISCHER: *Z. angew. Chem.*. **1929**, 1025; WICHMANN, *et al.: J. Assoc. Off. Agr. Chem.*, **1934**, 108.

form to be run either by hand or power finds widespread use in the determination of fat in milk (page 129). For many purposes, however, a machine of greater capacity and capable of higher speeds is highly desirable. Such a machine, capable of carrying loads up to 1,000 grams and giving a speed up to 3,000 r.p.m.,
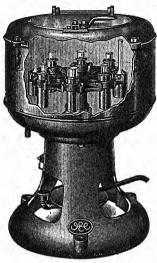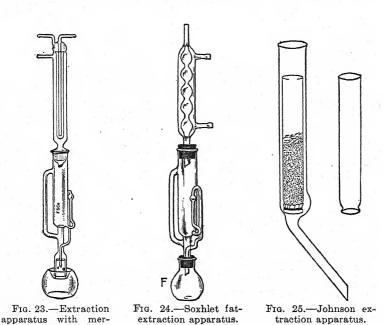
FIG. 22.—Electric centrifuge. (*Courtesy of Arthur H. Thomas Co.*)

is shown in Fig. 22. This machine, with enclosed rheostat, and embodying certain suggestions of the writer, which fit it especially for the requirements of food analysis, taking containers from Babcock bottles to 250-cc. cups,. will be found eminently satisfactory in the food laboratory. It can also be obtained mounted on a portable vibrationless stand for movement from place to place.

Extraction Methods.—For such determinations as fat in cereals or volatile oil in spices, where the substance is slowly removed by a proper solvent, some form of continuous extraction apparatus is essential. The one most commonly employed is the Soxhlet extractor, as shown in Fig. 24, its method of use being obvious. The connection between the weighed flask F and the extractor, as well as the upper connection, may be made by a soft, well-chosen cork. There is, however, more or less danger that resinous matter may be extracted from the corks by the solvent employed, so that it is preferable to cover them closely with tin foil. Both corks are sometimes replaced by ground joints, of which the lower, at least, should be standard and interchangeable. If there is no objection to having mercury exposed in the room, the form shown in Fig. 23, provided with an upper ground-glass joint and dipping into a pool of mercury in the flask, gives an air-tight seal. Notice that there should be two. holes in the neck of the flask above the mercury level to permit the return of the solvent to the flask. An improved but more complicated form of this apparatus with the extractor permanently sealed

to the condenser has been described by Walter and Goodrich.[1] All these special forms of Soxhlet apparatus are more fragile and more expensive to replace if broken. The Soxhlet apparatus has the disadvantage that it is very wasteful of the solvent, at least 50 or 60 cc. being ordinarily required for the extraction to proceed at all, and as commonly used the extraction is made by a cold solvent, which is often less desirable.

FIG. 23.—Extraction apparatus with mercury seal. (*Courtesy of Fisher Scientific Co.*)

FIG. 24.—Soxhlet fat-extraction apparatus.

FIG. 25.—Johnson extraction apparatus.

In many cases, where the quantity of material to be extracted is small, as in spices and cocoa, an advantageous form is the Johnson extractor, described by Winton in Leach-Winton, "Food Inspection and Analysis" and shown in Fig. 25. This has the advantage that the connection with the extraction flask is made by a small cork and also that only a small amount of ether, usually 10 cc., is needed so that it can be used by large classes or for numerous determinations without the labor of preparing large

[1] U. S. Dept. Agr., *Bur. Chem., Circ.* **69.** See also BENNINGTON: *Ind. Eng. Chem., Anal. Ed.,* **1932,** 125.

quantities of anhydrous ether. The fact that very small amounts of solvent are used is of help also when this is evaporated, since the evaporation takes less time and there is less danger of loss of

FIG. 26.—Underwriters extraction apparatus.

volatile oil, which is not the case when large quantities of ether must be evaporated.

The piece of linen tied to the bottom of the inner tube, as shown in the illustration, may with advantage be replaced by a small circle of filter paper held in place by a tapered glass collar.

A form of apparatus that combines many of the advantages of the Soxhlet and Johnson extractors and has the added merit of compactness, where a number of them are to be set up, is the so-called "Underwriters" extraction apparatus[1] shown in Fig. 26.

As a source of heat the free flame must be avoided on account of the inflammable nature of the solvents usually employed. A steam bath may be used or a water bath heated by electric immersion heaters, or the

small electric heaters shown in Fig. 27 are especially convenient for individual installations. It should be borne in mind, however, that solvents such as ether or petroleum ether should not be evaporated in any quantity in an open dish even on the electric heater, since some forms of these will ignite the vapors.

FIG. 27.—Electric hot plate.

Method.—The dried material is placed in an "extraction thimble" or wrapped in several thicknesses of paper and placed in the tube of the extractor, which is then connected with the condenser. A plug of cotton should be placed in the top of the thimble to prevent the pulverized sample from floating out as the solvent rises and being carried into the flask during the siphoning. Sirupy or viscous materials may be dried on some absorbent substance in a tin-foil or thin aluminum dish, which is then crumpled up and placed in the extractor. The needed quantity of solvent is then placed in the weighed flask which is joined to the apparatus. The time of heating may be from

[1] *Ind. Eng. Chem.*, **1914**, 78.

2 to 20 hours, varying with the material and the solvent. The heating should be such that the solvent siphons over about five or six times an hour or, in the case of the percolating type, such that a drop of solvent falls on the material about every 5 seconds.

After the extraction is complete the thimble is removed in the case of the siphon extractor, the apparatus again connected, and the flask heated again until the tube of the extractor is nearly filled with the solvent which may thus be recovered. The remainder is evaporated on the steam bath and the flask and contents dried in the oven to constant weight.

Choice of Solvent.—Extraction of food materials is ordinarily made with either anhydrous ethyl ether or petroleum ether—*i.e.*, a low-boiling distillate from petroleum (best between 35 and 45°C.). Of these two the petroleum ether is the cheaper and requires no special preparation beyond a possible fractionation to secure material boiling within the desired limits. It also has the advantage that it is not affected by traces of moisture that may be present in the material to be extracted and does not take up moisture during the extraction.

Ethyl ether, on the other hand, has the advantage that it is a better solvent for the fat than is petroleum ether, and most of the existing standards for food analysis are based on the use of this solvent. It has the disadvantage that it must be specially freed from water and kept so during the determination, since moist ether will dissolve sugar and other material that should not be included in the true ether extract.

Preparation of Anhydrous Ethyl Ether.—Wash ordinary ether with several portions of distilled water to remove alcohol, and allow it to stand with solid potassium hydroxide or calcium chloride until apparently all the water has been removed. Decant or filter into a dry bottle and remove the last traces of moisture by adding pieces of *clean*, freshly cut sodium. Allow the ether to stand over the sodium until there is no further evolution of hydrogen, keeping it protected from the moisture of the air by a stopper carrying a calcium chloride tube. The ether may be drawn off with a pipette as wanted for use.

Determination of Nitrogen.—Nitrogen determinations in foods are practically always made by some form of the Kjeldahl or moist combustion process. This method, which was originally

devised by Kjeldahl[1] for the determination of nitrogen in beers, is based upon the decomposition of the nitrogenous material by boiling it with strong sulphuric acid. The carbon and hydrogen of the material are oxidized to carbon dioxide and water, a portion of the sulphuric acid being reduced to sulphur dioxide, which is the actual agent for reducing the nitrogenous compounds. The nitrogen is left as ammonium sulphate from which the ammonia is liberated by potash or soda and distilled into a known excess of standard acid.

The original method of Kjeldahl has been modified at different times by the use of reagents designed to act as carriers of oxygen and thus facilitate the oxidation. Of these the two that have been most widely used are the modification of Wilfarth,[2] in which mercuric oxide is used as the oxygen carrier, and the Gunning method,[3] in which the boiling point of the sulphuric acid is raised by the addition of potassium sulphate. For ordinary foods either of these methods is satisfactory, but in the presence of alkaloids and certain other classes of organic compounds not all of the nitrogen is obtained.

The method described here is applicable in all cases and is practically the official "Kjeldahl-Gunning-Arnold" method of the Association of Official Agricultural Chemists.[4] It is really the Dyer[5] modification of the Kjeldahl method, since no copper salt is employed as catalyst.

Digestion.—Weigh ordinarily 0.7 to 3.5 grams of the sample, according to its nitrogen content, into a 500- to 600-cc. long-necked Kjeldahl flask, preferably of Jena or Pyrex glass. Care should be taken not to get particles of the material on the neck of the flask. It can be added through a narrow trough of glazed paper or conveniently by placing the weighed sample in a small piece of clean filter paper, folding it over and introducing the whole into the flask. Add 15 to 18 grams of potassium sulphate or anhydrous sodium sulphate, 0.7 gram of mercuric oxide or 0.65 gram of mercury, and 25 cc. of pure concentrated sulphuric acid, free from nitrogen. Place the flask in an inclined position

[1] *Z. anal. Chem.*, **1883**, 366.

[2] *Chem. Zentr.*, **1885**, 16, 113; *Chem.-Ztg.*, **9**, 502.

[3] *Z. anal. Chem.*, **1889**, 188.

[4] "Official Methods of Analysis," **1935**, p. 25.

[5] *J. Chem. Soc.*, **1895**, 811.

and close its mouth with a small glass funnel. Support the flask on a piece of asbestos board with a hole cut in it of such a size that the flame of the burner strikes only the portion of the flask below the level of the acid. Heat with a low flame until frothing ceases, and then boil briskly. Rotate the flask several times during the heating. The operation should be conducted in a hood with a good draught.[1] Boil until the mixture is colorless or nearly so, and then half as long again. In favorable cases the digestion may be completed within 2 hours, but if
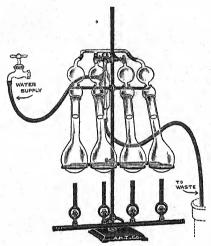
Fig. 28.—Fumeless digestion apparatus. (*Courtesy of Arthur H. Thomas Co.*)

alkaloids or other substances that are decomposed with difficulty are present, as in pepper, the boiling should be continued for at least 3 hours after the liquid has become clear.

Distillation.—After the digestion is finished allow the flask to cool somewhat, add cautiously 150 cc. of water, cool, and add 50 cc. of 4 per cent potassium sulphide solution (sodium sulphide solution of the same strength or 8 per cent sodium thiosulphate

[1] Sy (*Ind. Eng. Chem.*, **1912,** 680) has described an ingenious apparatus (Fig. 28) by which the digestion can be made directly in the laboratory, the fumes of sulphuric acid being drawn from the flask and dissolved in a stream of water. Other forms, which hang in the mouth of the digestion flask and prevent the escape of acid fumes by absorption, are also available. (HENWOOD and GAREY: *J. Franklin Inst.*, **221,** 531; CASSIDY: *Ind. Eng. Chem., Anal. Ed.*, **1937,** 478.)

solution may be used equally well), and mix thoroughly to precipitate the mercury, which might otherwise hold back part of the ammonia as mercur-ammonium compounds. Add 70 cc. of cold saturated sodium hydroxide solution,[1] pouring it carefully down the side of the flask so that it shall not immediately mix with the acid and liberate the ammonia. Add about a gram of granulated zinc or zinc dust, which by the evolution of hydrogen prevents bumping, a piece of paraffin the size of a pea to prevent

FIG. 29.—Kjeldahl connecting bulb. (*Courtesy of Arthur H. Thomas Co.*)

frothing, and at once connect the flask with the condenser by means of a connecting bulb, Fig. 29. The condenser tip should dip into a measured amount (25 to 40 cc.) of $0.1N$ hydrochloric or sulphuric acid in the receiver. With larger amounts of nitrogen $0.25N$ or even $0.5N$ acid may be used. Mix the contents of the flask by shaking and distill about 150 cc., taking care to rinse the delivery tube free from the acid solution at the end of the distillation. Titrate the excess of acid with $0.1N$ alkali, using methyl red or cochineal as indicator.[2] For laboratories in which many routine nitrogen determinations are to be made, compact multiple digesting and distilling units, using either gas or electricity for heating, can be obtained from the dealers.

Notes.—Where occasional determinations are to be made and one is not bound to the official method, the writer much prefers to carry out the distillation by means of steam, using an apparatus as shown in Fig. 30, the time of distillation being in this way much shortened and no bumping occurring. Whatever method of distillation is employed, connection between the condenser and the flask containing the alkaline solution should be made by some efficient form of "spray trap," to prevent fine particles of alkali being carried over mechanically with the steam into

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[1] Approximately 450 grams of NaOH, free from nitrates, in a liter of water. Specific gravity should be 1.43 to 1.48.

[2] *Methyl Red.*—Dissolve a gram of the dry indicator in 50 cc. of 95 per cent alcohol, dilute to 100 cc. with water, and filter if necessary.

Cochineal.—Digest 3 grams of pulverized cochineal in a mixture of 50 cc. of 95 per cent alcohol and 200 cc. of water for one or two days, shaking frequently, and filter.

the acid in the receiver. The one shown in Fig. 30 is made from an ordinary 100-cc. distilling flask, or the form shown in Fig. 29, specially made for the purpose, and of large capacity, may be used.

In the steam distillation the use of zinc and paraffin is not necessary, except that the latter is sometimes required in the case of milk. Potash can also be used in place of sodium hydroxide if desired, since the presence of the separated sulphate, which causes the bumping in direct distillation, is not objectionable in this case.

Copper sulphate (0.3 to 1.0 gram of the crystals, or the equivalent amount of copper turnings) can be used in place of the mercury if desired. In this case the addition of the sulphide solution is not necessary. There are a few organic nitrogen compounds, however, with which mercury is a better metallic catalyst than is cop-
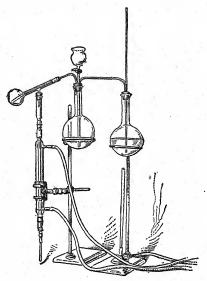
FIG. 30.—Kjeldahl distilling apparatus.

per. Low results may be obtained if a considerable excess of sulphuric acid is not present during the digestion.

Selenium, either alone or with some other agent as mercury or copper, has been recommended as a catalyst.[1] Its use has aroused much comment, favorable and otherwise; but the consensus of opinion seems to be[2] that selenium alone has no special advantage over mercury or mercuric oxide for difficult digestions, but that, if added to the mercury catalyst, the time of digestion is shortened, in general approximately one-half, often being completed in 30 to 45 minutes. In favorable cases the solution clears in 20 to 30 minutes and an afterboil one-half as long completes the digestion. The amount of selenium added is usually 0.1 gram

[1] LAURO: *Ind. Eng. Chem., Anal. Ed.*, **1931**, 401.

[2] SANDSTEDT: *Cereal Chem.*, **1932**, 156; TAYLOR: *Ind. Eng. Chem., Anal. Ed.*, **1933**, 263; OSBORNE and KRASNITZ: *J. Assoc. Off. Agr. Chem.*, **1934**, 339.

of the element itself.   With the great majority of food materials the shortening of the digestion time and the elimination of the sulphide precipitation might well warrant its use in spite of the somewhat greater cost.

Another simple and useful variation from the established methods is the collection of the ammonia in a solution of boric acid[1] as described in the simplified method below.   The ammonia may be changed quantitatively from borate to chloride in the presence of the slightly ionized boric acid.   The method is advantageous in that only one standard solution need be used, the risk of a possible change in the standard base is avoided, and the absorbing reagent need not be accurately measured.

The reagents used should be tested when a new lot is prepared. This is best done by making a blank determination, using 0.5 gram of cane sugar, which will aid in the reduction of any nitrates that may be present in the reagents.

Simplified Method.—The method described on page 38 is a general and official one, applicable to practically all food products. In most cases, however, the following simplifications will be equally satisfactory.   Transfer about 0.5 to 1.5 grams of the finely divided substance to a 300-cc. digestion flask, add 25 cc. of concentrated sulphuric acid free from nitrogen, to which has been added 20 grams of selenious oxide (SeO_2) per liter.[2]   If preferred 0.05 to 0.1 gram of powdered selenium may be added with the sodium sulphate instead of dissolving the oxide in the sulphuric acid.   Add 8 grams of sodium sulphate and place a small funnel in the neck of the flask, which should be supported in an inclined position on wire gauze and heated with a small flame under a hood or with suitable provision against the escape of acid fumes until frothing has ceased and the liquid boils quietly, which usually requires half an hour.   Then increase the heat and boil the solution for 15 to 30 minutes after it clears.

Measure 25 cc. of 0.1N acid from a burette into a 300-cc. Erlenmeyer flask and place the condenser tip (Fig. 30) beneath the surface of the liquid, adding a little water, if necessary, to seal it.   If preferred, 50 cc. (measured with a graduate) of a 5 per cent (saturated) solution of boric acid may be substituted for

[1] WINKLER: *Z. angew. Chem.,* **1913,** 231.

[2] The technical grade is entirely satisfactory and much cheaper than the purified material (obtainable from Eimer & Amend, New York).

the standard acid. Care must be taken in this case, however, that an efficient condenser be used so that the boric acid solution shall be cold at all times.

Transfer the digested sample by means of three 25-cc. portions of distilled water to the distilling flask of the apparatus and connect the flask with the condenser. Add an excess (60 to 75 cc.) of saturated sodium hydroxide solution through the separatory funnel and distill off the ammonia by steam. A few drops of phenolphthalein may be used to show when enough sodium hydroxide has been added, remembering that much excess of alkali destroys the pink color. When 150 cc. have distilled over, remove the collecting flask, after rinsing off the condenser tip with distilled water, and titrate the excess of acid with 0.1N sodium hydroxide; or, if boric acid has been used, titrate the ammonia directly with the standard acid, using preferably methyl red as indicator in either case.

Determination of Nitrogen in the Presence of Nitrates.—Occasionally it may be required to determine total nitrogen in a sample that contains some nitrates as well as organic nitrogen compounds. By the methods just described there would be loss of nitric acid when the sulphuric acid is added for the digestion. This may be avoided by adding the acid in the presence of some substance, as phenol or salicylic acid, which is readily nitrated and thus holds the nitric acid as a nitro-derivative. The nitro-compound is then reduced and the determination carried out as before.

Method.[1]—To the sample, weighed as before, add 30 cc. of sulphuric acid containing 1 gram of salicylic acid, pouring the acid on quickly and at once covering the sample with it. Allow it to stand with frequent shaking for at least 30 minutes, or until complete solution results; then add 5 grams of crystallized sodium thiosulphate and heat over a low flame until all danger of frothing has passed. Increase the heat and boil briskly until white fumes no longer escape from the flask (5 to 10 minutes). Add approximately 0.7 gram of mercuric oxide or its equivalent in metallic mercury and continue the boiling for $\frac{1}{2}$ hour after the liquid in the flask has become colorless or nearly so. If the contents of the flask are likely to become solid before this point is reached, add 10 cc. more of the sulphuric acid.

[1] Assoc. Off. Agr. Chem., "Official Methods," **1935**, p. 25.

Cool, dilute, and distill as in the Kjeldahl-Gunning-Arnold method on page 38.

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CHAPTER II

THE MICROSCOPICAL EXAMINATION OF FOODS

The Value of the Microscope in Food Analysis.—With many classes of food materials, such as spices, cocoa, coffee, and cereal products, the examination for adulterants is far from complete unless the sample has been examined with the microscope. This is because the microscope often reveals much more clearly than does chemical analysis the nature of the adulteration. For instance, the chemical analysis of a sample of cocoa showed that the amount of starch present was somewhat greater than usually corresponds to the other constituents, but left the analyst in doubt as to whether the amount was greater than could still be explained by the possible natural variation in starch content of genuine cocoa, or implied added starch. The microscope, however, in this particular case showed at a glance the presence of a distinct amount of arrowroot starch, a form that differs so markedly from the starch of the cocoa bean as to be readily distinguished from it. It would surely not be too much to say that in detecting certain forms of adulteration the microscope is the main reliance of the food analyst. If to this we add the ease and quickness with which the examination can be made and the fact that by comparing different microscopic fields with mixtures of known composition it is often possible to approximate the percentage of the adulterant present, the great value of the microscope for this work becomes apparent; hence a certain amount of practice in its use should always accompany a course in food analysis.

Students who have had the advantage of an elementary laboratory course in plant histology will find this distinctly helpful, although the absence of such preliminary training should not deter anyone from undertaking the microscopic study of foods. The microscopic structures by which the common adulterants are recognized are comparatively simple and a reasonable expenditure of time and study should enable the student to gain sufficient

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knowledge of the microscopical examination to supplement to great advantage the chemical analysis. To obtain the best results the work should be carried out systematically, and much time will be gained if it can be done under the direction of a competent instructor.

Apparatus.—*a. Essentials.* *Microscope.*—The first requisite is a suitable microscope. A thoroughly satisfactory modern instrument can be purchased for about $80 (Fig. 31).[1] It should be provided with a double nosepiece carrying two objectives of 16- and 4-mm. equivalent focus and giving, with a medium (10×) eyepiece, magnifications of 100 and 450 diameters. A numerical aperture of 0.65 for the higher power objective, with its accompanying greater working distance, will be found admirably adapted for food work, and the objectives, as will be the case with modern lenses, should be par-focal and accurately centered so that no time will be lost in changing from one to the other.

This, together with a suitable number of the ordinary glass microscope slides and cover glasses, the latter preferably ¾-in. circles, make up the absolute essentials of the apparatus needed.

FIG. 31.—Microscope for food analysis. (*Courtesy of E. H. Sargent & Co.*)

An Abbe condenser, N.A. 1.20, with iris diaphragm attached, can be added for about $10 more and is a decided convenience in securing sufficient illumination on dark days, but it is not absolutely essential.

b. Accessories.—Of these, the most useful will be an *eyepiece micrometer* and a *micropolariscope*. The micrometer is used to measure the size of starch grains, stone cells, etc. In its simplest

[1] A second-hand instrument in good condition and entirely satisfactory can usually be purchased from dealers in used apparatus in New York City or elsewhere.

form it consists of a disk of glass, bearing a scale usually graduated in 0.1 mm., which rests on the diaphragm of the eyepiece so that the scale is viewed superimposed upon the object as seen in the microscope. The exact value of the scale divisions depends upon the objective used and can be ascertained approximately from tables in the catalogs of the principal makers or measured directly by means of a stage micrometer.

The micropolariscope is used in the examination of starches, some of which exhibit distinct differences when viewed in this way, and in examining fats, such as butter, to tell whether or not they have been crystallized. It is an accessory that would probably find more general use if the microscope makers would provide some means of applying it quickly and conveniently. A common form consists of a polarizer mounted so as to be used in the substage ring above the mirror, and an analyzer which may be screwed to the microscope tube above the double nosepiece or to the inner draw tube as preferred. A much cheaper and entirely satisfactory substitute may be found in commercial "Polaroid." A "Polaroid-Junior" attachment consisting of a polarizer placed on the stage below the object slide, and a rotating graduated analyzer that fits over the ocular may be obtained for about $3.

If expense is no deterrent, a so-called "chemical microscope" of the Chamot type, as made by Spencer Lens Company or Bausch and Lomb, practically a simple form of petrographical microscope, is far preferable for examinations by polarized light and can be used equally well with ordinary light, although quite a little more expensive than the simpler form illustrated.

A *demonstration eyepiece*, showing a small pointer in the field, is useful in calling attention to some definite object. One can be easily improvised by cementing a short firm hair by means of a drop of mucilage to the diaphragm of the eyepiece in such a way that it projects nearly to the center of the field. By moving the slide it is easy to bring to the end of the pointer any particular object to which it is desired to call attention. For the preparation of permanent mounts and thin sections for study a *turntable* (Fig. 32) and *sectioning razor*, a heavy razor concave-ground on one side only, will be found useful. Use a piece of material, not too thick, previously softened by soaking if necessary, and held in the fingers or between pieces of pith

or cork.   A safety-razor blade in a simple holder is eminently satisfactory.

Preparation of the Sample.—The sample will frequently, as in the case of spices, breakfast cocoa, etc., be in a sufficiently fine condition to examine directly.   If quite coarse like coffee, it may be necessary to grind a small portion of it, which may be done conveniently in a small porcelain mortar.   Samples like chocolate, which contain a large amount of fat or oil, may be treated several times on a small filter with portions of ether and then dried on the steam bath.   The residue obtained after the treatment with sulphuric acid and alkali in the determination of crude fiber (see page 304) is often good hunting ground for stone cells and other hard tissues.   A bit can be taken from the moist residue on
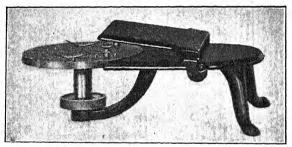
FIG. 32.—Turntable for making permanent mounts.

the point of a knife and examined under the microscope without appreciably affecting the weight.

Temporary Mounts.—The most serviceable method is to mount the sample directly in water.   A bit of the powder is placed on the center of the slide, a drop of water placed near it, and the powder scraped into the water with the edge of the cover glass. It is then covered with the glass and rubbed gently between the thumb and finger until the material seems to be evenly distributed and free from coarse or gritty particles.   It is best not to put too much on the slide because only a confused mass will be seen through the microscope.   Enough water should be used so that the cover will lie flat on the slide, any excess being removed at the edge of the cover glass with the torn edge of a bit of filter paper.

Reagents.—For the greater part of the work the plain water mount as just described will be sufficient, but occasionally it may

be desired to treat the sample with some reagent to bring out more distinctly characteristic structures, or to render the sample clearer and free from debris. The two reagents most useful are dilute iodine solution and chloral hydrate. The first of these is of great value in showing the presence of starch, especially when present in very small amount. It can be added very easily to the temporary water mount by placing a drop at the edge of the cover and touching the opposite edge with a bit of filter paper. Some of the water is withdrawn by capillary attraction, and thus a portion of the iodine solution is brought into contact with the material and its action can be observed. It should not be used if the starch is to be identified, since the deep color imparted to the grain will obscure many of its characteristics.

For dissolving the starch and other cell contents and rendering the harder tissues more transparent for examination the chloral hydrate may be used. A large drop of a 60 per cent solution is placed on the slide, the powder added and covered loosely with a cover glass. The slide is then heated carefully over a small flame until the liquid boils gently, and then quickly cooled by placing it on a cold surface. If necessary another drop of the solution can be drawn under the cover glass and the process repeated.

Permanent Mounts.—It is sometimes desired to mount samples of especial interest so that they will be permanently available. This may be done by using either glycerin jelly or Canada balsam as a mounting medium instead of water.

In mounting a sample in glycerin jelly a bit of the jelly[1] is placed on a slide and warmed over a small flame until melted, a little of the powder added to it, and the cover glass, previously warmed to prevent enclosure of air bubbles, gradually lowered onto the melted drop and gently pressed down. The excess of jelly which exudes can be removed when the slide is cold.

For examination by polarized light the best mountant is Canada balsam. A drop of the balsam dissolved in xylol is placed in the center of the slide, the material added, and the whole covered with a warm cover glass. After standing for several days the

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[1] One part of gelatin by weight is soaked in 6 parts of water, 7 parts of glycerin are added and finally 1 per cent of phenol. The mixture is warmed and stirred for 10 or 15 minutes until clear, and filtered hot. Or it may be purchased already prepared.

balsam will become very hard and the excess can be scraped off with a knife or dissolved in xylol or chloroform.

Some of the starch grains, mounted in this way and examined with crossed Nicols, exhibit characteristic and striking phenomena (see page 55). It should be noted in passing, however, that balsam slides of starches are not of much use for examination with ordinary light because the refractive indexes of balsam and starch are so nearly the same. Permanent mounts in balsam should in general have the tissues cleared (freed from water by successive dehydrating agents) and stained.

If it is desired to preserve the mounts in glycerin jelly for any great length of time the cover glass should be sealed to prevent the jelly from drying out by evaporation. This may be done by
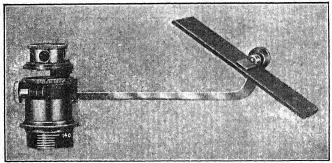
Fig. 33.—Camera lucida.

placing a ring of cement or asphalt varnish around the cover so that it is partly on the cover and partly on the slide, using the turntable and a pointed camel's hair brush. It should be said, however, that by far the best results are obtained with freshly prepared slides.

Authentic Samples for Comparison.—It is essential that the student should have at hand a collection of samples representing the pure spices or other products which are being examined, together with the common adulterants. A complete set, in the case of the spices for instance, would comprise a collection of the whole unground spices in order that their general characteristics may be noted and the relations of the different parts studied with a hand lens; thin sections of the various spices cut longitudinally and transversely and mounted as permanent slides for study with

the microscope are also of great value, showing oftentimes the structures more clearly to the beginner than in the powdered sample, and giving as well the relative positions of the different

tissues; finally, specimens of the powders themselves should be included, for the purpose of making temporary mounts and comparing directly with the material in question.

Such authentic samples can be prepared without much trouble by the student himself by powdering small quantities of the whole spices, or they may be purchased together with the prepared sections at slight cost.[1] The laboratory should possess a systematic collection of those in most common use ready for immediate access. Known mixtures for testing the proficiency of the student at each point in his progress will also be found helpful.

Permanent Records.—If it is desired to preserve for future reference or study any particularly interesting specimen it can be drawn by means of a camera lucida (Fig. 33), the details being filled in freehand, or it may be photographed.
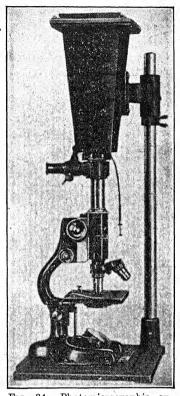
Fig. 34.—Photomicrographic apparatus.

Comparatively simple and self-contained apparatus for photomicrography can be obtained, and the process, after conditions have once been fixed, is a simple routine method.

Figure 34 shows a simple form of photomicrographic camera which is well suited for making a photographic record of observations since it is quite automatic and the observation eyepiece

[1] "Southalls Standard Powders for Analysts" will be found a useful collection, and many collections put out to illustrate works on materia medica contain spices and other food products as well as drugs.

permits the field to be examined and photographed without change of position of the operator.

Typical Plant Tissues.—The vegetable materials used as food are often from different parts of the plant and would naturally be expected to present differing structural features under the microscope. Among the spices, for instance, ginger is a root, cassia a bark, allspice a berry, cloves are flower buds. A study of these under the microscope shows, however, that many of the
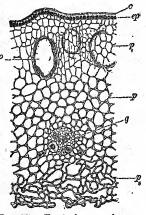
Fig. 35.—Typical parenchyma, p_1, p_2, p_3. (*Moeller*.)

plant tissues are repeated in the different parts so that the number that are of principal importance is not so great; as a matter of fact, the features that serve as guides or marks of identification to the food microscopist are comparatively few in number and quite easily recognized.

The most important of them are:

Parenchyma.—This is the name given to the thin-walled cellular structure of the softer portions of the plant. The cells are usually more or less regular in outline and may or may not still show the cell contents. Typical examples are shown at p_1, p_2, and p_3 in Fig. 35. A characteristic thick-walled parenchyma is shown in Fig. 132, page 596.

Stone Cells.—These occur most frequently in the protective portions of the plant, in the shells of nuts, in the bark, and in the coatings designed to protect the delicate seeds. They are irregular in shape and differing in color, but have in general thickened walls and very prominent radial cracks extending through the walls. With polarized light and crossed Nicols they show brilliant interference colors on account of double refraction. (See Figs. 36 and 37 for characteristic forms.)

Bast Fibers.—Bast fibers are of the same general nature as stone cells, but differing in that they are very long in proportion to their width, with tapering pointed ends and a very narrow central canal. They are most commonly found in the fibrovascular elements or sap-conducting tissues of the plant. Distinctive forms of these are to be seen in Fig. 38.

Ducts and Vessels.—The fibrovascular bundles contain also various forms of vessels or ducts, which are thin-walled tubes
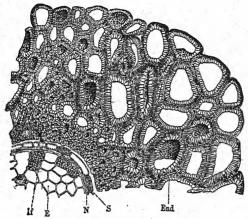
Fig. 36.—Stone cells, *End.* (*Winton.*)
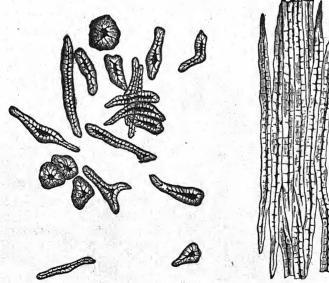
Fig. 37.—Stone cells from the shell of the coconut. (*Winton.*)

Fig. 38.—Typical bast fibers. (*Vogl.*)

variously known according to the appearance of the thickenings in the walls as *annular, spiral, scalariform,* or *reticulated* ducts.

In the softer portion of the bundle occur *sieve tubes*, peculiar thin-walled cells with porous partitions known as *sieve plates* (see Fig. 39 and Fig. 128, *a*, page 595).

Cell Contents.—Of the important features of the cell contents two should be noted here—*starch* and *resin*. The starch is, upon the whole, the chief reliance of the food microscopist in the identification of many adulterants on account of the wide differences in the size and shape of the granules, which will be discussed in greater detail below.
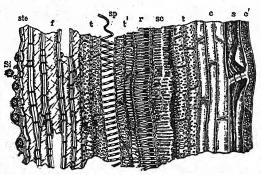
Fig. 39.—Fibrovascular bundle from the mesocarp of the coconut; *t* tracheids with small pits; *t'* tracheids with large pits; *sp* spiral vessel; *r* reticulated vessel; *sc* scalariform vessel; *s* sieve tube. (*Winton.*)

The resins, from their striking color, are at times of great service in indicating the presence of adulterants. Typical examples are allspice and Bombay mace.

The appearance of these characteristic structures can be shown clearly in prepared sections of the plant. Viewed in this way they can be pointed out to the student more readily than when a powder, consisting of a more or less confused mass of disintegrated tissues, is examined under the microscope. A list of such prepared sections which the writer has found helpful is:

Parenchyma.—Elder pith (transverse and longitudinal sections), coffee.

Stone Cells.—Pepper, pericarp of allspice.

Bast Fibers.—Clove stems, cassia (longitudinal sections).

Vessels and Ducts.—Chicory, ginger, *Pteris*, squash.

Starch in situ.—Wheat, bean.

Resins.—Bombay mace.

Starches.—The ability to differentiate the various starches and recognize them under the microscope is of great importance. Water mounts of the pure starches should be made and studied until the student can recognize any in a mixture. The points to be noted are, especially, size, shape, markings, groupings, presence or absence of a hilum and its position in the granule. Examination by polarized light, noting the presence of polarization crosses with dark field, Nicols crossed, and bright colors with a selenite plate, may also be helpful. Instead of the selenite a cleavage of mica (muscovite) about 0.05 mm. in thickness, slipped under the specimen, may be used. It should of course be above the polarizer (polaroid) if this is used on the stage and should be turned 45° from extinction, *i.e.*, until dark, then 45° more. In any case it is best to rotate the specimen if possible, the polarizer and analyzer still remaining crossed. Care should be taken to choose a portion of the slide where the grains are not crowded too closely together, and a preparation mounted in balsam is best for examination by polarized light.

It should be borne in mind in examining the starches that not all the grains in a given microscopic field will be of the exact size or shape that are characteristic of a particular starch. For instance, wheat starch is characterized as a circular starch with a central hilum. In a given field there will be some grains that are oval or lens-shaped and that show no hilum. Such individual cases should be neglected and attention paid to the majority of the granules. It is true also that accidental contamination may be found with one or a few grains of a different starch. Such should be disregarded and those taken into consideration which appear to be present in the greatest proportion and in every field.

Possibly the simplest classification of the starches is that depending upon their general shape into circular, oval, elliptical, and polygonal. The common ones would be divided under this method into:

Circular.—Wheat, rye, barley, tapioca.

Oval.—Potato, arrowroot, sago.

Elliptical.—Pea, bean.

Polygonal.—Corn, oats, buckwheat, rice.

1. Circular Starches. *Wheat Starch.*—The grains are irregularly circular in outline, or where they are tipped up on edge they may appear lens-shaped. There are two principal sizes of gran-

ules, the larger, which are more characteristic, varying in size from 30 to 40μ,[1] and the more numerous smaller ones, averaging about 5μ. The larger grains usually show a small central dotted hilum, and at times concentric rings, especially by oblique light. The appearance by polarized light is not especially characteristic (Fig. 83, page 588).

Rye Starch.—This starch is of the same general character as wheat starch, comprising two sizes of granules. The larger grains are somewhat greater in size than the corresponding grains of wheat, a considerable proportion being over 50μ in diameter. The concentric rings are rather more prominent, and the hilum frequently shows slight cracks radiating from it (Fig. 84, page 588).

Barley Starch.—The granules of barley starch closely resemble those of wheat but are somewhat smaller, seldom being over 35μ in diameter (Fig. 85, page 588).

Tapioca Starch.—This starch is smaller than those that have just been described, averaging about 15 to 20μ, the grains being fairly uniform in size. They are in general quite round and are characterized by many of the grains being truncated or shaped like a kettledrum. The hilum is usually a distinct central dot, and the rings are not ordinarily seen (Fig. 86, page 588).

2. Oval Group. *Potato Starch.*—This is a typical oval starch and the largest of the common starches. The grains average 60 to 80μ in diameter with very pronounced rings, the so-called "oyster-shell markings." The hilum is a dot at the smaller end of the granule. With crossed Nicols the polarization crosses are very showy, and with a selenite or mica plate brilliant colors are observed (Figs. 87 and 88, page 588).

Arrowroot Starch.—There are several varieties of starch known under the name of arrowroot, but the term is here limited to the West India product. This starch resembles potato in a general way, but the grains are somewhat smaller, seldom over 50μ, and the hilum is usually a transverse fissure at the larger end of the granule. It often shows two wings, as in the conventional sign for a bird flying. The rings are quite distinct and the polarization effects very pronounced (Fig. 89, page 589).

[1] The unit of measurement in microscopical work is the *micron*, denoted by the Greek letter μ, and occupying one-thousandth of a millimeter (0.001 mm.).

Sago Starch.—This is another of the larger starches, the grains averaging 40μ in size and individuals being occasionally 80μ in length. In shape they are irregularly oval, the larger ones often showing one or more protuberances with flattened surfaces, indicating contact with other grains in aggregates which have been broken up in the process of manufacture. The hilum is toward one side of the granule and is frequently cracked. The rings are distinct, and characteristic crosses are shown by polarized light (Fig. 90, page 589).

3. Elliptical Group. *Pea Starch.*—The starches of the pea and the bean are very similar and typical of the leguminous starches in general. They are ellipsoidal in shape, averaging about 50μ in length, with distinct rings. The hilum is usually a pronounced cleft with numerous lateral branches. The polarization crosses are ⤬ shaped (Fig. 92, page 589). The bean starch is shorter and more rounded, as well as smaller than the pea (Figs. 91 and 93, page 589).

4. Polygonal Starches. *Corn Starch.*—This is the largest and most common of the polygonal starches, some grains being 30μ in diameter, though 20 to 25μ is more usual. The grains sometimes occur in groups of three or more, but more commonly singly. The hilum is centrally placed and often shows radiating cracks (Fig. 94, page 589).

Buckwheat Starch.—This starch is somewhat similar to corn but is not so sharply angular, is somewhat smaller, ranging in size from 6 to 12μ, and the hilum is less pronounced and does not show the radiating cracks. Very characteristic, however, is the grouping into polygonal masses. Both the individual starch grains and the aggregates are shown in Fig. 95, page 590.

Oat Starch.—The oat starch belongs also to the polygonal group. The granules are comparable in size with those of buckwheat, although somewhat smaller, seldom exceeding 10μ in diameter. Like buckwheat, too, they form aggregates, which are usually round or elliptical in shape. Occasional grains are long and spindle-shaped which is fairly characteristic of this starch (Fig. 96, page 590).

Rice Starch.—Rice is the smallest of the common polygonal starches, the grains averaging about 5μ in diameter. Masses of grains occur sparingly, not nearly so commonly as in buckwheat or oat starch. The individual grains are quite sharply

angular, this and the size serving to distinguish them from the other members of the polygonal group. The hilum and rings are seldom seen (Fig. 97, page 590).

Typical Food Products.—The examples given in the following pages have been selected as being well fitted to give the student an idea of the forms of adulteration to be detected by the microscope, and to show its value. The list is by no means complete, and no attempt has been made to make it so. Some of the spices, as allspice and ginger, are included for their value in microscopical training although not discussed chemically. The special advantage of the synopsis given for each product is that the beginner's attention is focused upon the tissues of prime importance from the analyst's standpoint, and he is not distracted by the minor details which are very properly given in the descriptions and figures that aim to show complete microscopical structure.

The proper procedure in each case is for the student, having become familiar with the prominent plant tissues through the examination of typical sections and studied the starches in water mounts, then to examine in systematic order powdered samples of the pure spice in question and its common adulterants, noting the important features as summarized in the synopsis. His proficiency should then be tested by examining and reporting upon several prepared mixtures whose composition is unknown to him. Having done this with a fair degree of accuracy, he is ready to examine the unknown sample with confidence.

The list of adulterants given in each case does not include everything that could possibly be used for adulteration, but things that have been variously reported as actually found. Reference should constantly be made to the authorities given in the list at the end of this chapter, especially to the works of Winton and Leach.

Allspice.—(Fig. 98, page 590.)

CHARACTERISTICS.

> *Starch.*—Small (av. diam. = 8μ), nearly circular, uniform in size, central dotted hilum (Fig. 98, *a*).
>
> *Stone Cells.*—Large, colorless, plainly marked, and quite numerous (Fig. 98, *b*).
>
> *Resin.*—Yellow, brown, or red lumps of waxy luster and striking appearance. Especially characteristic (Fig. 98, *c*).

Adulterants.

Clove Stems.—Figure 99, page 590; see also Fig. 110, page 592.

Stone cells, similar to those of allspice; bast fibers, allspice has none (Fig. 110, *a*); vascular ducts, especially characteristic (Fig. 99, *a*; Fig. 110, *b*).

Nutshells.—Figure 100, page 590.

Stone cells are usually long and spindle-shaped, yellow-brown in color with brown contents; bits of colorless trachea (Fig. 100, *a*).

Fruit Stones.—Figure 101, page 591.

Masses of long, colorless stone cells resembling those of coconut shells but free from color.

Cayenne.—Figure 102, page 591.

Outer-skin of fruit pod, usually reddish-brown in color with a cellular structure, *a*; epidermis of seed is greenish-yellow with very peculiar and characteristic markings resembling the convolutions of the intestines, *b*.

Pepper.—For the characteristic structures of pepper in greater detail, see page 62. It is most readily recognized in mixtures by the characteristic polygonal masses of starch grains (Fig. 124, page 594).

Turmeric.—See Mustard, page 62.

Ginger.—Figure 116, page 593; see also page 61.

Starch oval, smooth, showing neither hilum nor rings, but many granules have characteristic small protuberances at one end, as at *a, a,* Fig. 120, page 594.

Pea Hulls.—Figures 103 and 104, page 591.

Pea starch (page 57) accompanied by aggregates of the long rectangular cells of the palisade layer, averaging 60 to 100μ in length (Fig. 104, *a*).

Cereals.—Recognized by the characteristic starches, page 55 *et seq.*

Cassia (Cinnamon).—Figure 105, page 591.

Characteristics.

Wood fibers, red-brown in color, often grouped in bundles with grains of starch interspersed (Fig. 105, *a*).

Bast Fibers.—Figure 105, *b.*

Stone cells, resembling somewhat those of allspice, but brownish in color, rather more oblong and usually with one wall distinctly thicker than the other (Fig. 105, *c*).

Starch.—The starch is quite similar to that of allspice, but often occurs as compound grains made up of two to four single grains. These may at first glance appear to be large single grains, but close inspection will show their compound nature.

Yellow patches of cellular tissue with starch grains showing through may be abundant.

ADULTERANTS.

Ground Bark.—The fibers from this resemble the woody fibers of cassia, but are coarser and usually do not show the starch grains interspersed that characterize the cassia tissues (Fig. 106, *a*, page 591).

Ginger.—Recognized best by the starch (see page 61).

Sawdust.—The long coarse fibers occur in bundles often crossed at right angles by the medullary rays (Fig. 107, *a*, page 592). Sometimes the fibers or tracheids show distinct openings or pores. See *t* and *t'*, Fig. 39, page 54.

Turmeric.—See Mustard, page 62.

Cassia Buds.—These have many tissues in common with cassia but may be distinguished by broader, shorter bast fibers and especially by short thick-walled crooked hairs (Fig. 108, *a*, page 592).

Cereals.—Distinguished by the characteristic starches.

Cloves.

CHARACTERISTICS.—Figure 109, page 592.

Confused mass of cellular tissue; few bast fibers.

ADULTERANTS.

Clove Stems.—Figure 110, page 592, and Fig. 99, page 590. Recognized by characteristic vascular ducts and stone cells as given under allspice.

Nutshells.—See Allspice, page 59.

Fruit Stones.—See Allspice, page 59.

Allspice.—The element best suited for showing allspice is the colored resin (Fig. 98, *c*, page 590, and Fig. 111, *a*, page 592).

Ginger.—See Allspice, page 59.

Cereals.—Recognized by their characteristic starches, page 55 *et seq.*

Cocoa.

CHARACTERISTICS.—Figure 112, page 592.

Starch.—Small, nearly circular, hilum central, dotted, rather indistinct; frequently occurs in twins or triplets (Fig. 112, *a*).

Pigment Cells.—Yellow, brown, or violet in color.

Dark miscellaneous debris of the cotyledons.

ADULTERANTS.

Cocoa Shells.—Figure 113, page 593. *Spiral ducts* may occur in long spirals, *a*; or simply as fragments showing only as a ring or half a link of a chain, *b*. Occasional fragments occur in the pure ground cocoa, on account of the difficulty of separating the shells completely in the process of manufacture, but their presence in distinct and recurring amounts is indicative of the presence of shells. Thick-walled *stone cells* (Fig. 114, *a*, page 593) are characteristic, but in general can be seen readily only after clearing the slide with chloral hydrate.

Cereals, etc.—Wheat, arrowroot, sago, corn. Distinguished by the characteristic starches, which are usually much larger than those of cocoa and easily recognized (see Fig. 115, page 593).

Ginger.

CHARACTERISTICS.

Starch.—Figure 116, page 593. Oval, flattened, often with a tapering rounded angle at the smaller end which serves to distinguish it from wheat, which it most nearly resembles. It is a large starch, the maximum size being about 45μ, while the average size is 25μ. The hilum and rings are indistinct and not of much assistance in identification. The starch is the most prominent feature of powdered ginger.

Scalariform Vessels.—Figure 117, *a*, page 593. Occasional wood fibers with starch grains showing through (Fig. 117, *b*).

ADULTERANTS.

Turmeric.—See Mustard, page 62.

Cayenne.—See Allspice, page 59, also Fig. 118, *a*, page 593.

Sawdust.—See Cassia, page 60. Note the distinction between the natural fibers of the ginger and the coarser

tracheids of ordinary sawdust, as shown at *a* and *b* in Fig. 119, page 594.

Cereals.—Characteristic starches. Figure 120, page 594, shows ginger adulterated with wheat and corn.

Mustard.

CHARACTERISTICS.—Figure 121, page 594.

General appearance is that of a confused mass of gray cellular tissues. No starch is present.

Mustard hulls, shown by bits of the yellow and brown palisade cell layer (Fig. 121, *a*) may appear here and there in the pure powdered mustard.

Epidermal Cells (Fig. 121, *b*).—Colorless, cellular, and mucilaginous.

ADULTERANTS.

Mustard Hulls.—The presence of the characteristic palisade layer in abnormal amounts, at times even exceeding the proportion of the regular tissues of the seed, or in large masses as in Fig. 122, page 594, is an indication of the admixture of hulls. Comparison should be made with the amount found in powdered genuine mustard flour.

Turmeric.—The bright yellow color of the "paste balls" is very characteristic. The starch is oval, large with very distinct "oyster-shell" markings, but fragile and easily broken up, so not of so great importance for identification (Fig. 123, *b*, page 594).

Cereals.—Identified by their characteristic starches, as at *a*, Fig. 123.

Pepper.

CHARACTERISTICS.—Figure 124, page 594; see also page 397.

Starch.—Individual grains are very small, averaging about 3μ in diameter. They are polygonal in shape and show a distinct central hilum. Of more importance for identification are the aggregates or polygonal masses of closely packed grains (Fig. 124, *a*).

Stone Cells.—These are yellow, very thick-walled, with a tendency toward square ends (Fig. 125, *a*, page 595).

Other elements, of less importance, but which occur occasionally, are groups of the cup-shaped "beaker cells," bits of

the brown or yellow parenchyma of the shell and needle-like crystals of piperin (Fig. 126, page 595).

ADULTERANTS.

Added Pepper Shells.—This is usually determined microscopically by the presence of an excessive number of stone cells of the shell. Comparison should be made with the number to be noted in several whole pepper corns ground in a porcelain mortar (Fig. 125, *a*, page 595).

Olive Stones.—See Allspice, page 59.

Nutshells.—See Allspice, page 59.

Turmeric.—See Mustard, page 62.

Long Pepper.—Characteristic disagreeable odor, more pronounced when heated. With polarized light and crossed Nicols, the starch masses show a glimmering white appearance quite different from that of true pepper. The appearance is a little more striking if the mount is made in glycerol.

Buckwheat.—Distinguished by the greater size of the starch aggregates and of the individual grains (Fig. 127, *a* and *b*, page 595).

Other Cereals.—Distinguished by their starches, page 55 *et seq.*

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CHAPTER III

FOOD COLORS AND PRESERVATIVES

COLORS

The addition of coloring matter to food products, apart from any question of the harmful nature of the color itself, may be objectionable or otherwise, depending upon the character of the food to which it is added and the object desired in the addition.

If the color is added simply to satisfy the esthetic sense of the consumer and does not in any way constitute a deception, there can be no serious objection to its use. In candy, for instance, the consumer is perfectly aware that the color is entirely artificial, and if the color used is harmless the practice is unobjectionable.

In other cases, however, color is added to conceal inferiority or to simulate an appearance of greater value. Such practices as the addition of yellow color to pastry to imitate the presence of eggs, or the injection of red color into watermelons to give the appearance of ripeness, are obviously fraudulent. In the majority of such cases the interests of the purchaser are sufficiently protected if the package is plainly marked to show the presence of artificial color. The use of artificial color in diluted orange juice drink, such as orangeade, simulating the real orange juice, has been held to be an adulteration that cannot be corrected by any form of labeling. It is obvious that the use of color which is in any way harmful should not be permitted under any circumstances.

At the present time, when only the permitted dyes are in general use because the greater demand has lowered their price, the question of color present is not so important, but has been retained because of its interest and educational value.

Kinds of Color Used.—The coloring matters employed in foods may be conveniently divided into three classes: coal-tar dyes, vegetable colors, and mineral colors. Of these the coal-tar colors are used to a greater extent than the others but subject to definite supervisory regulations.

Preliminary Treatment for Vegetable and Coal-tar Colors.— Many products, as sirups, candies, jams, and other saccharine foods, may be taken up directly in hot water and strained, if necessary. Liquids containing much alcohol should be evaporated on the water bath until the alcohol is removed. Solid materials, as fruits, meat products, macaroni, etc., are best digested with 70 per cent alcohol, containing from 3 to 5 per cent of ammonia. The filtered solution is heated on the water bath until the alcohol and ammonia have been removed, made faintly acid with acetic acid, and tested by dyeing on wool as described on page 70. In the case of macaroni and similar pastes, to avoid the glutinous mass that will coat the wool, it is best to evaporate the alcoholic ammoniacal extract containing the coloring matter until most but not quite all of the alcohol is removed. The thick liquid is then treated with one-half its volume of concentrated hydrochloric acid and poured into a large separatory funnel. Amyl alcohol equal to about two-thirds of the original volume of the solution is added, and sufficient 25 per cent salt solution to make the mixture separate well. The color dissolves in the amyl alcohol and may be freed still further from the glutinous protein by washing the alcohol layer a few times with salt solution acidified with hydrochloric acid.[1] The amyl alcohol may be treated further as described on page 73. For other methods of separating the color from such products see page 99.

Unless the experience of the analyst or previous knowledge of the character of the product tested is sufficient to indicate the nature of the color likely to be employed, it is best to proceed for the identification of the color systematically as described below.

Systematic Examination of a Food Product for Color.— In the examination of a product suspected to have added color present, it is usually best, both on account of the comparative ease with which the test can be carried out on an impure solution or suspension of the material, and because of the greater frequency with which the acid coal-tar dyes are employed, to make first the dyeing test on wool as described on page 70. Especial care should be taken to note the possible presence of a mixture of coal-tar colors.

This may be done by fractional dyeing as described on page 71 or by Goppelsroeder's method, which consists in allowing the end

[1] *Assoc. Off. Agr. Chem.,* "Official Methods," **1935**, p. 79.

of a long strip of filter paper supported vertically to dip into the aqueous or alcoholic solution of the color. After standing for several hours observe whether the paper shows along its length zones of different hues. In the case of powdered products the presence of color mixtures may be shown by blowing a little of the powder from the tip of a spatula horizontally over a sheet of moistened filter paper. Note if the colored spots that dot the wetted paper all show the same color.

If a mixture of colors is found it will generally be advisable to separate the colors by a systematic procedure as given on page 91. The possibility of a basic color being present should not be overlooked.

If a coal-tar color is not found or if the color of the material cannot all be accounted for by the presence of such a color, vegetable color should be sought. This may be conveniently separated from the material by extraction with amyl alcohol and the color identified by the tables and special tests on pages 95 to 100. It will usually be necessary to test for only a limited number of the vegetable colors, cochineal for example being hardly to be expected in a lemon extract, or turmeric in currant jelly.

If the presence of artificial color is still suspected and none has been found up to this point, there still remain the pigments or mineral colors, and the examination of some classes of food materials for added color would not be complete unless search had been made for this group of colors, usually by testing the ash for aluminum, tin, etc.

In the case of coal-tar colors, it should be borne in mind that the tables given in this chapter include only a selected list of colors so that one may be found which does not coincide in all its reactions with any of those tabulated. The permitted dyes are naturally the ones that will be found of most frequent occurrence.

Coal-tar Colors.—Although the number of coal-tar dyes is very great, Mulliken[1] listing some 1,500 individuals, the number that is used in food products is comparatively limited. On account of the use to which they are put in foods, they are for the most part readily soluble in water, and fortunately for the analyst, most of them are *substantive*, dyeing directly on wool without requiring the use of a mordant. The greater number are "acid"

[1] "Identification of Pure Organic Compounds," Vol. III.

dyes, *i.e.*, they will dye wool from a faintly acid solution, while a few are "basic" dyes, being best taken up by the wool from a slightly alkaline bath.

The following list includes, with the exception of a few oil-soluble colors, which for convenience are grouped together in a supplementary list, practically all the coal-tar dyes that have met with any application in coloring foods. Since they have at different times been listed in government regulatory announcements in two ways, both are given here. The numbers in parentheses at the left are the numbers of the dyes in Schultz and Julius, "A Systematic Survey of the Organic Colouring Matters," edited by A. G. Green, while the numbers in brackets refer to the colors as listed in the 1924 "Colour Index" of the Society of Dyers and Colourists of England, which gives the composition of these dyes, and the names in parentheses are common synonyms for the same dye.

(398)[5]	Naphthol Green B.
(1)[7]	Picric Acid.
(3)[9]	Naphthol Yellow (Martius Yellow, Manchester Yellow).
(4)[10]	Naphthol Yellow S (Martius Yellow S, Acid Yellow S).
(8)[16]	Fast Yellow G (Fast Yellow, Acid Yellow).
(13)[26]	Croceine Orange (Brilliant Orange, Ponceau 4GB).
(14)[27]	Orange G.
(43)[63]	Orange GT (Crocein Orange Y).
(53)[77]	Palatine Scarlet (Brilliant Cochineal 2R).
(55)[79]	Ponceau 2R (Xylidine Red, Xylidine Scarlet).
(56)[80]	Ponceau 3R (Cumidine Red, Cumidine Ponceau).
(65)[88]	Fast Red B (Bordeaux B).
(64)[89]	Crystal Ponceau (Crystal Scarlet 6R).
(71)[114]	Azoeosine.
(95)[138]	Metanil Yellow.
(87)[142]	Orange III (Methyl Orange, Helianthine).
(88)[143]	Orange IV (Tropæolin 00, Diphenylamine Orange).
(89)[144]	Brilliant Yellow S.
(84)[148]	Resorcin Yellow (Tropæolin Yellow, Chrysoine).
(85)[150]	Orange I (Naphthol Orange).
(86)[151]	Orange II (Gold Orange, Mandarin G).
(102)[176]	Fast Red A.
(103)[179]	Azorubin S (Fast Red C, Carmoisin).
(107)[184]	Amaranth (Fast Red D).
(106)[185]	Brilliant Scarlet (New Coccin, Cochineal Red A).
(108)[186]	Ponceau 6R (Scarlet 6R).
(160)[277]	Croceine Scarlet 3B (Ponceau 4RB).
(163)[280]	Biebrich Scarlet (Fast Ponceau B, Ponceau 3RB).

(169)[286]　Croceine Scarlet 7B.
(240)[370]　Congo (Congo Red).
(277)[448]　Benzopurpurine 4B.
(278)[449]　Benzopurpurine 6B.
(287)[463]　Azo Blue.
(269)[480]　Chrysamine R (Chrysamin).
　(94)[640]　Tartrazine.
(425)[655]　Auramine.
(427)[657]　Malachite Green (New Green, Fast Green).
(428)[662]　Brilliant Green (Ethyl Green).
(433)[666]　Guinea Green B.
(435)[670]　Light Green SF Yellowish (Acid Green).
(448)[677]　Mangenta (Fuchsine, Aniline Red).
(451)[680]　Methyl Violet B (Methyl Violet DB).
(452)[681]　Crystal Violet.
(462)[692]　Acid Magenta (Acid Fuchsine).
(464)[695]　Acid Violet 4BN.
(468)[698]　Formyl Violet 4BS (Acid Violet 4B Extra).
(476)[703]　Methyl Alkali Blue.
(480)[707]　Soluble Blue (Water Blue).
(440)[712]　Patent Blue VN (New Patent Blue B, 4B).
(439)[715]　Cyanol Extra (Acid Blue 6G).
(504)[749]　Rhodamine B.
(510)[766]　Uranine (Fluorescein).
(512)[768]　Eosine (Eosine A).
(517)[773]　Erythrosine.
(518)[774]　Phloxine P.
(520)[777]　Rose Bengal.
(523)[779]　Rose Bengal 3B.
(667)[801]　Quinoline Yellow (Chinoline Yellow).
(584)[841]　Safranine.
(585)[842]　Methylene Violet 2RA.
(601)[861]　Induline Soluble (Fast Blues).
(602)[865]　Nigrosine Soluble.
(650)[922]　Methylene Blue (Methylene Blue B, NG).
(692)[1180]　Indigo carmine (Indigo Disulphoacid).

Oil-soluble dyes:

　　(7)[15]　Aniline Yellow.
　(16)[19]　Butter Yellow.
　　　[22]　Yellow A.B.
　(10)[23]　Sudan G.
　(11)[24]　Sudan I.
　　　[61]　Yellow O.B.
(143)[248]　Sudan III.
　　　　　　Oil Orange SS.
　　　　　　Oil Red XO.

Permitted Dyes.—With a view to restricting the coal-tar colors used in foods to those whose harmlessness is beyond question, it is permitted under Federal food legislation to use such color in certain cases, but only definite specified colors may be employed. The list of these "permitted colors" has been varied from time to time, but at the present writing the list includes the following dyes, the numbers on the left having the same significance as on page 67. Names not preceded by numbers are new dyes not yet listed in the "Colour Index." The common name of the dye is given in each case, and the name in parentheses at the right is the new name adopted in the regulations enforcing the Food, Drug and Cosmetic Act. The designation "FD&C," in distinction from names having only "D&C" or "C," means that the color may be used in foods and is not restricted to drugs and cosmetics, or to cosmetics alone.

Red shades:

> (56)[80] Ponceau 3 R (FD&C Red No. 1).
> (107)[184] Amaranth (FD&C Red No. 2).
> (517)[773] Erythrosine (FD&C Red No. 3).
> Ponceau SX (FD&C Red No. 4).
> Oil Red XO (FD&C Red No. 32) (oil-soluble color).

Orange shades:

> (85)[150] Orange I (FD&C Orange No. 1).
> Orange SS (FD&C Orange No. 2) (oil-soluble color).

Yellow shades:

> (4)[10] Naphthol Yellow S (FD&C Yellow No. 1).
> Naphthol Yellow S—Potassium Salt (FD&C Yellow No. 2).
> [22] Yellow AB (FD&C Yellow No. 3) (oil-soluble color).
> [61] Yellow OB (FD&C Yellow No. 4) (oil-soluble color).
> (94)[640] Tartrazine (FD&C Yellow No. 5).
> Sunset Yellow FCF (FD&C Yellow No. 6).

Green shades:

> (433)[666] Guinea Green B (FD&C Green No. 1).
> (435)[670] Light Green SF Yellowish (FD&C Green No. 2).
> Fast Green FCF (FD&C Green No. 3).

Blue shades:

> Brilliant Blue FCF (FD&C Blue No. 1).
> (692)[1180] Indigotine (Indigo Disulphoacid) (FD&C Blue No. 2).

These may be used under definite regulations[1] as to their purity and mode of manufacture, the dyes being expected in general to correspond to the chemical formula specified in the regulation and to be free from objectionable or poisonous substances introduced during manufacture or in the intermediates employed. Under the regulations of the Food, Drug and Cosmetic Act, "certified dyes" only can be used, the designation signifying that the manufacturer has taken special precautions to obtain a pure product and that representative samples of each lot of dye have been submitted to the Food and Drug Administration of the U. S. Department of Agriculture. Exhaustive tests having shown that the color corresponds to "specifications," a certificate is issued to the manufacturer for that particular lot of dye.[2]

Although they were originally distinctly higher in price than the ordinary coal-tar dyes, the demand for these certified colors has been such that they are the ones commonly used at present. It should be clearly understood, however, that when the evident purpose of the dye is to conceal inferiority or damage, even certified dyes cannot be used, although declared on the label.

Detection of Coal-tar Colors.—In the detection of a coal-tar color in food two methods are commonly employed to isolate the color: (1) dyeing on wool, and (2) extraction with immiscible solvents.

Dyeing on Wool. *Procedure.*—If the material is a liquid, use about 50 cc. directly; if a solid, about 25 grams should be mixed with water as thoroughly as possible and made up to a volume of approximately 100 cc. If the solution is not already acid, add a drop of hydrochloric acid (sp. gr. 1.12) or enough to produce a very slight but distinct acid reaction. Add a piece of white woolen cloth about 2 in. square, which has been thoroughly washed in boiling water, and boil in the colored solution for at least 10 minutes, replacing the water lost by evaporation. Remove the wool and if colored rinse thoroughly in boiling water to remove any color which may be adherent to the fiber. Strip the color from the wool by boiling with dilute ammonia (1 part of strong ammonia to 50 parts of water). Remove the wool, add

[1] See "Federal Register," May 9, 1939, and Sept. 16, 1939.
[2] Federal Security Agency, Food and Drug Administration. Service and Regulatory Announcements, Food, Drug and Cosmetic, No. 3.

dilute hydrochloric acid to the solution until it is faintly acid, immerse a fresh piece of the woolen cloth and boil again for 10 minutes. In general, a distinct color on the second piece of wool indicates the presence of a coal-tar dye.

Notes and Precautions.—It is not necessary to have a perfectly clear solution of a solid material, although it should not be so thick as to bump badly when boiled.

The size of the piece of cloth used for dyeing should be governed somewhat by the amount of color present. If only a trace of color is evident, less wool should be employed in order that it shall be as deeply dyed as possible. This applies perhaps more strongly to the second dyeing than to the first, since the color can be collected from the original solution on a fairly large piece of wool and then concentrated on a smaller piece in the second dyeing.

Care should be taken that the dye bath is not too strongly acid. Some of the colors, Naphthol Yellow S for example, do not dye well in a strongly acid bath and hence might be overlooked. Enough only should be added to give a slight but distinct reddening to blue litmus paper. In many cases, as in fruit products, the food itself will contain sufficient organic acid without adding the hydrochloric acid. When only small amounts of color are present it is often best to obtain a fairly clear solution and evaporate nearly to dryness on the water bath with a trace of acid and a small piece of wool. For the subsequent identification of the dye a piece of wool only 1 in. square, dyed to a full shade, is better than a much larger piece merely tinged with color.

An essential point is to watch the progress of the dyeing in the first bath. It is best to use a succession of rather small pieces of cloth, removing one as soon as it seems to have taken up what color it will, then adding another, and so on until the color is exhausted from the solution. In this way it may usually be determined whether more than one color is present, the different dyes often dyeing the wool at unequal rates. The actual separation of the colors may be carried out in the same way, although larger amounts can usually be obtained for identification more readily by separation with immiscible solvents as described on page 72.

If the only purpose is to detect the presence of a coal-tar dye, the single dyeing and stripping outlined above will ordi-

narily suffice. If, however, the dyed wool is to be used for identifying the color, especially if vegetable color is also present, a further stripping and dyeing is advisable in order that the color may be as pure as possible.

While the dyeing method is the simplest and in many respects the best method for detecting coal-tar colors, and it is amazing to see the readiness with which colors are removed relatively pure from the heterogeneous mass that may be found in many food products, its limitations should be borne in mind. The method as described is not suited for basic dyes, and if these are suspected the method should be reversed, the dyeing being done in weak ammonia solution (1 part of strong ammonia to 50 of water) and the stripping made by dilute acetic acid (5 cc. of glacial acid to 100 cc. of water). Basic dyes are less commonly found in foods than are the acid colors.

Archil and cudbear dye wool by this procedure in a manner similar to the coal-tar colors but can be recognized readily by the purple color of the wool when stripping with the ammonia, and by the fact that the dyed fiber is decolorized by zinc and hydrochloric acid, the color being restored on exposure to the air (see also page 97). Indigo carmine is changed by stripping with ammonia, hence may not be found in the second dyeing. If there appears to be a blue color present which dyes the first piece of wool but not the second, a piece of the first dyeing should be tested for indigo, as on page 82.

The vegetable colors (except archil and cudbear) give practically no color in the second dyeing by this test, although if very large amounts are present some may persist, and it may be necessary to strip and redye several times. The presence of a vegetable color can frequently be predicted by reason of the marked change in color of the wool when treated with ammonia.

Extraction with Solvents.—Although not of so general application as the dyeing test described above, extraction with solvents is sometimes employed when somewhat larger quantities of color are to be separated for identification. The most suitable solvent is amyl alcohol and the method may be outlined as follows:

Prepare an aqueous solution of the colored material by macerating and straining or filtering if necessary, make it alkaline with sodium hydroxide and shake with amyl alcohol in a separa-

tory funnel, carefully avoiding the formation of an emulsion. Test a little of the amyl-alcohol layer for basic colors by shaking it with dilute acetic acid in a test tube. If the lower layer is colored, a basic or weakly acid dye may be present and the main portion of the amyl alcohol should be evaporated to dryness on a water bath, adding a little ethyl alcohol from time to time to hasten the evaporation. Take up the residue in hot water and dye the color on wool from a bath faintly ammoniacal in the case of basic dyes, and acid with a drop of acetic acid with weakly acid dyes.

The alkaline solution from which the basic colors have been removed is made strongly acid with one-half its volume of hydrochloric acid (sp. gr. 1.20) and again shaken with amyl alcohol and 2 or 3 cc. of ethyl alcohol. Most of the acid colors will be dissolved by the amyl alcohol, although it may not be deeply colored itself. A few of the highly sulphonated colors will still remain in the aqueous layer. The amyl alcohol is drawn off and shaken with dilute sodium hydroxide to remove the color. The aqueous layer is separated, acidified, and the color dyed on wool as described on page 70. If the amyl alcohol still shows some color, it can usually be removed by adding an equal volume of petroleum ether and again shaking with sodium hydroxide. It should be borne in mind that the amyl alcohol will dissolve vegetable colors, so that the presence of coal-tar dyes should not be assumed unless shown by a double dyeing of the color extracted by the amyl alcohol.

Oil-soluble Colors.—An alcoholic solution of the color can usually be obtained by shaking the oil or melted fat with an equal volume of 90 per cent alcohol and washing the alcoholic solution several times with gasoline to remove traces of the fat. The alcoholic solution of the dye can be used for further tests.

Identification of Coal-tar Dyes.—Although the identification of a coal-tar color is much more difficult than its simple detection in a food, the examination of a product for color can hardly be considered complete unless this be done.

The first essential is to obtain from the material, by the methods just mentioned, as large a quantity of the dye in as pure a condition as possible, both dyed on wool and in aqueous solution. It may be possible to do this very easily, as in the case of confectionery, in which the color is frequently in the form of a

surface coating readily removed by a slight washing with water. With other foods the color may be extracted by a suitable solvent and obtained pure by several washings and reextractions. If the most available method of separating the color seems to be by dyeing on wool, a solution for testing may be obtained by stripping a portion of the dyed wool with dilute ammonia, evaporating the ammoniacal solution to dryness on the water bath and taking up the residue in a little water.

The color may be identified by testing either the dyed wool or the aqueous solution and it is usually best to use both methods. The tests should not be applied haphazard but in a definite order.

Since the tests given are based on the reactions of chemical individuals it is necessary that before applying them to mixtures of colors these should first be separated into their components as described on pages 91 to 94 before applying the systematic procedure.

Identification by Tests on the Aqueous Solution.—The identification of the color by the systematic application of color reactions and solubility tests has long been practiced, but the tables in ordinary use for this purpose, such as those of Weingärtner,[1] or Rota[2] will not be found of much value in food work since they require in many cases considerably larger quantities of color than can usually be obtained from a food product. Of much greater assistance is the table of solubilities and the detailed scheme proposed by Loomis.[3] Even this is based on the behavior of 0.01 per cent solutions of the dyes, a distinctly stronger solution than is available in many cases, so that with more dilute solutions the reactions described are not always characteristic. In Table 4[4] the tests have been planned to identify colors which are present in concentrations of 0.01 and 0.002 per cent. The primary division into blue and violet, red, yellow and orange, and green colors is based on the color dyed on wool in a faintly acid bath. This simple scheme does not differentiate the similar dyes Ponceau 2R and Ponceau 3R; Orange III and Metanil Yellow. The oil-soluble dyes are grouped at the end of the table.

[1] *J. Soc. Dyers and Colourists*, **1887**, 67.

[2] *Chem.-Ztg.*, **1898**, 437.

[3] U. S. Dept. Agr., *Bur. Chem. Circ.* **63.**

[4] HAUB, HATTIE D. F., Thesis, Mass. Inst. Technol., 1912.

TABLE 4.—ANALYTICAL SCHEME FOR IDENTIFICATION OF COAL-TAR DYES BY TESTS, MAINLY ON THE AQUEOUS SOLUTION

In adding the reagents such as NaOH and HCl, a drop is added at a time. Usually the first drop gives some results. In trying any test on the dyed fiber, a piece of the dyed cloth is wet with distilled water and used for comparison so that a brightening of the color due to moisture will not be ascribed to the action of the reagents. In most cases the reactions on the dyed fiber are not used as a means of differentiating the dyes, since good sharp color distinctions are obtained only with the deeply dyed fiber, while the reactions with NaOH, HCl, and concentrated H_2SO_4 also hold for the more dilute solutions.

Unless stated otherwise, all tests are made on small portions (2 to 5 cc.) of the aqueous solution. This aqueous solution may be obtained by extracting the color with amyl alcohol, as on page 72, evaporating and dissolving in water, or, in the case of most colors, by stripping the dyed wool with ammonia, evaporating to dryness on the water bath to expel ammonia, and dissolving in a little water.

Tests on the "dry dye" are made by evaporating 2 to 5 cc. of the aqueous solution in a small porcelain dish, placing 3 to 4 drops of strong sulphuric acid alongside the spot, and streaking it over the dye with a glass rod. After noting the color and rubbing up the dye spot with the acid, 10 cc. of water is added and any change in color observed.

If in using the scheme a dye is reached which does not agree in its confirmatory tests with the unknown sample, the procedure should be carefully traced back to make sure that an error in judgment at some point has not placed the unknown in the wrong group.

Tests on the oil-soluble dyes are made on the neutral alcoholic solution. Silk, instead of wool, is used for tests on the dyed fiber.

A. Blue and Violet Dyes

Add concentrated H_2SO_4 to dry dye

1. BLUE.—Add HCl to aqueous solution.
   - *a.* Violet. — Nigrosine Soluble (602)[865]
   - *b.* No change.
     Add 10 per cent NaOH to aqueous solution.
     Crimson. — Azo Blue (287)[463]
     Green. — **Indigotine** (692)[1180]
2. RED.
   - Aqueous solution blue. — Methyl Alkali Blue (476)[703]
   - Aqueous solution violet. — Soluble Blue (480)[707]
3. PALE YELLOW OR COLORLESS.—Treat dyed fiber with 10 per cent NaOH.
   - Greenish yellow to yellow. — Cyanol Extra (439)[715]
   - Bluish green to green. — Patent Blue VN (440)[712]
   - No change. — **Brilliant Blue FCF**

4. PURPLE OR VIOLET.—Add 10 per cent NaOH to aqueous solution.

    Violet.   Induline Soluble (601)[861]

    Green.   **Indigotine** (692)[1180]

5. GREEN.

    Aqueous solution red violet, no   Methylene Violet 2RA

      change on dilution.   (585)[842]

    Aqueous solution blue, greenish

      blue on dilution.   Methylene Blue AD (650)[922]

6. ORANGE.—To 5 cc. of aqueous solution, add 1.5 cc. 10 per cent NaOH and 5 cc. of ether and shake 30 seconds. Separate water completely from ether and wash latter twice with 2 cc. of water + 2 drops of 10 per cent NaOH, separating water completely each time. Finally, shake ether with 2 cc. of water and 1 cc. of 25 per cent acetic acid.

  a. Color of acetic acid approximately equal to original color. Add 10 per cent NaOH (1 drop to each 2 cc.) to aqueous solution and shake with ether.

    Ether straw yellow.   Methyl Violet (451)[680]

    Ether colorless.   Crystal Violet (452)[681]

  b. Color of acetic acid only slight fraction of original color. Compare color of very dilute aqueous solution with known dyes.

    Blue violet.   Acid Violet 4BN (464)[695]

    Red violet.   Formyl Violet 4BS (468)[698]

B. Red Dyes

I. Dilute aqueous solution strongly fluorescent. Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with an equal volume of ethyl acetate.

  1. Extract has pink fluorescence.   Rhodamine B (504)[749]

  2. Extract colorless.

    Add concentrated H_2SO_4 to dry dye.

    a. Becomes orange, colorless on

      dilution.   Phloxine P (518)[774]

    b. Becomes yellow, orange red on

      dilution.   Eosine (512)[768]

II. Dilute aqueous solution not fluorescent. (In 0.01 per cent solution Erythrosin shows faint fluorescence which decreases with dilution.) Add concentrated H_2SO_4 to dry dye.

1. CRIMSON OR RED.

  Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with ethyl acetate.

    a. Much color extracted.   Azoeosine (71)[114]

　　b. Almost no color extracted.　　　Palatine Scarlet (53)[77]
　　　　　　　　　　　　　　　　　　　　Xylidine Red (55)[79]
　　　　　　　　　　　　　　　　　　　Ponceau 3R (56)[80]
　　　　　　　　　　　　　　　　　　. **Ponceau SX**
　　Distinguish by careful tests on dyed fiber and comparison with
　　known colors.

2. PURPLE OR VIOLET.
　　Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for
　　every 2 cc.), with amyl alcohol.
　　　a. Color nearly all extracted.
　　　　Treat dyed fiber with concentrated HCl.
　　　　　(1)　Blue.　　　　　　　　Croceine Scarlet 3B (160)[277]
　　　　　(2)　Red.
　　　　　Treat dyed fiber with NH₄OH.
　　　　　(*a*)　Violet.　　　　　　Archil (710)[1242]
　　　　　(*b*)　Pink.　　　　　　　Fast Red A (102)[176]
　　　　　　　　　　　　　　　　　Azorubin S (103)[179]
　　　b. Almost no color extracted.
　　　　　(1)　Dry dye with concentrated H_2SO_4 gives *red* violet.
　　　　　　　　　　　　　　　　Brilliant Scarlet (106)[185]
　　　　　(2)　Dry dye with concentrated H_2SO_4 gives *blue* violet.
　　　　　　　(*a*)　Color of dry dye is violet.　**Amaranth** (107)[184]
　　　　　　　(*b*)　Color of dry dye is red brown.　Ponceau 6R (108)[186]

3. BLUE.
　　Add HCl to the aqueous solution.
　　　a. Becomes blue.　　　　　　Congo Red (240)[370]
　　　b. Becomes purple.　　　　　Benzopurpurine 4B (277)[448]
　　　　　　　　　　　　　　　　　Benzopurpurine 6B (278)[449]
　　　c. Practically unchanged.
　　　　To aqueous solution add one-fifth volume of 10 per cent NaOH
　　　　　and shake with ether.
　　　　　(1)　Extracted.　　　　　Safranine (584)[841]
　　　　　(2)　Not extracted.
　　　　　　　(*a*)　Dyed fiber violet red.　Bordeaux B (65)[88]
　　　　　　　(*b*)　Dyed fiber orange red.
　　　　　　　Treat dyed fiber with concentrated HCl.
　　　　　　　Violet.　　　　　　Crystal Ponceau (64)[89]
　　　　　　　Greenish blue.　　　Croceine Scarlet 7B (169)[286]

4. ORANGE.
　　Add 10 per cent NaOH to aqueous solution.
　　a. Decolorized.

Shake aqueous solution acidified with HCl (1 drop of 1.12 acid for every 2 cc.), with amyl alcohol.

    (1) Color in upper layer.   Magenta (448)[677]

    (2) Color in lower layer.   Acid Magenta (462)[692]

b. No change.   **Erythrosine** (517)[773]

    Rose Bengal (520, 523)[777, 779]

Compare carefully with known samples. Rose Bengal is pink in aqueous solution, Erythrosine is red orange. If enough pure color is available test for Cl and I.

5. GREEN.   Biebrich Scarlet (163)[280]

C. Yellow and Orange Dyes

I. Aqueous solution has intense green fluorescence.

Color all extracted by acidified amyl alcohol.   Uranine (510)[766]

II. Aqueous solution not fluorescent.

Add concentrated H_2SO_4 to dry dye.

    1. COLORLESS.

    Add HCl to aqueous solution and shake with ethyl acetate.

    All extracted.   Picric Acid (1)[7]

    Practically none extracted.   Auramine (425)[655]

    2. ORANGE OR YELLOW.

      a. Yellow.

      Add HCl to aqueous solution.

      (1) No change.   **Tartrazine** (94)[640]

      (2) Decolorized.

      Shake with ethyl acetate, separate from aqueous layer, wash once with a little water and shake with dilute NH_4OH.

      (*a*) NH_4OH colored yellow. Naphthol Yellow (3)[9]

      (*b*) NH_4OH colorless.   **Naphthol Yellow S** (4)[10]

      (3) Becomes orange.   Fast Yellow G (8)[16]

      b. Orange.

      Add NaOH to aqueous solution.

      (1) No change.

      Dry color and dyed wool orange

          Orange GT (43)[63]

      Dry color and dyed wool yellow.

          Quinoline Yellow (667)[801]

      (2) Becomes redder.

      Dyed wool is yellow.   Resorcin Yellow (84)[148]

      Dyed wool is orange red.   Croceine Orange (13)[26]

          Sunset Yellow FCF

3. CRIMSON OR RED.
 Crimson.   Orange G (14)[27]
 Orange red.   Orange GT (43)[63]

4. PURPLE OR VIOLET.
 a. Red violet.
  Add HCl (1.12) to aqueous solution and shake with ethyl acetate.
   Much color extracted.   Orange II (86)[151]
   No color extracted.   Brilliant Yellow S (89)[144]
 b. Blue violet.
  Add HCl (1.12) to aqueous solution.
  (1) Decolorized.   Chrysamine R (269)[480]
  (2) Not decolorized.
   Add NaOH to aqueous solution.
   (*a*) Becomes brown red.   **Orange I** (85)[150]
   (*b*) No change.   Orange III (87)[142]
            Orange IV (88)[143]
            Metanil Yellow (95)[138]
  (In 0.01 per cent solution NaOH gives a white precipitate with Orange III on standing.)

D. Green Dyes

Add concentrated H_2SO_4 to dry dye.

1. BLUE.   Nigrosine Soluble (602)[865]

2. YELLOW OR ORANGE.
 Shake color solution, acidified with HCl and saturated with NaCl, with an equal volume of acetone.
 a. Color mainly in acetone layer.
  Color of dry dye and dyed wool is blue green.
            Malachite Green (427)[657]
  Color of dry dye and dyed wool is yellow green.
            Brilliant Green (428)[662]
 b. Color mainly in aqueous layer.
  Add 10 per cent NaOH to aqueous solution.
  (1) Becomes purple.   **Fast Green FCF**
  (2) No change.   Napthol Green B (398)[5]
  (3) Decolorized.   **Guinea Green B** (433)[666]
           Light Green S F Yellowish (435)[670]
  (Compare reactions of dry dye and dyed fiber carefully with known samples, also extraction by amyl alcohol from solution faintly acid with acetic acid.)

E. Oil-soluble Colors

Since some of the members of this group, as Yellow AB and Yellow OB, FD&C Orange No. 2 and FD&C Red No. 32, differ very slightly from each other in constitution, the final tests should be made carefully and known dyes always used for comparison.

Add concentrated H_2SO_4 to dry dye.

1. ORANGE OR YELLOW.
   Add HCl to alcoholic solution.
a. No change.	Sudan G (10)[23]
b. Becomes orange.	Aniline Yellow (7)[15]
c. Becomes red.	Butter Yellow (16)[19]

2. PURPLE OR VIOLET.
   a. Dyed fiber red. Sudan III (143)[248]
   b. Dyed fiber orange.
   Shake 5 cc. of a neutral gasoline solution of the dye with 5 cc. of a mixture of 1 part 40 per cent formaldehyde (formalin) and 4 parts acetic anhydride. Note color of lower layer after 30 seconds. (Compare with known samples.)
(1) Red.	**Yellow AB** [22]
(2) Orange.	**Yellow OB** [61]

3. RED.
   Treat the dyed silk with concentrated HCl.
   a. Orange red. Sudan I (11)[24]
   b. Violet.
Red violet.	**Orange SS FD&C No. 2**
Blue violet.	**Red XO FD&C No. 32**

   (Compare with known samples.)

The identification of a color by the systematic tests described in Table 4 should not be regarded as conclusive until it has been confirmed by tests on the dyed fiber as described in the following table (Table 5).[1]

The tests are carried out, using the four reagents named, on small pieces of the dyed cloth on a porcelain tile or in small white porcelain dishes. The colors are noted after the reagent has acted for about 30 seconds. Two results are given in each case, for the different intensities of color obtained with the dye solutions as described on page 74. The numbers directly preceding the dye have the same significance as on page 67.

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[1] A more extended table of the same character is given by Mathewson, U. S. Dept. Agr., *Bull.* **448,** pp. 37–45.

TABLE 5.—COLOR REACTIONS OF DYED FIBER
A. Violet and Blue Colors

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(287)[463] *Azo Blue:*				
0.01	No change	Blue	Red	Red violet
0.002	No change	Greenish blue	Pink	Pink violet
(451)[680] *Methyl Violet:*				
0.01	Brownish yellow	Brownish yellow	N. decolor.	Paler
0.002	Faint brown	Pale brown	Decolor.	Decolor.
(452)[681] *Crystal Violet:*				
0.01	Orange	Orange	Partly decolor.	Partly decolor.
0.002	Pale yellow	Yellow	Decolor.	Decolor.
(464)[695] *Acid Violet 4BN:*				
0.01	Deep yellow	Orange	N. decolor.	Much fainter
0.002	Yellow	Yellow	Decolor.	Much fainter
(468)[698] *Formyl Violet 4BS:*				
0.01	Yellow	Deep orange	N. decolor.	Lighter
0.002	Pale yellow	Orange	Decolor.	Lighter
(476)[703] *Methyl Alkali Blue:*				
0.01	Greenish blue	Red brown	N. decolor.	Lighter
0.002	Faint greenish blue	Red brown	Decolor.	Lighter
(480)[707] *Soluble Blue:*				
0.01	Bluer	Red brown	Red brown	Decolor.
0.002	Greenish brown	Brown	Decolor.	Decolor.
(440)[712] *Patent Blue VN:*				
0.01	Yellow	Green yellow	Greener	No change
0.002	Pale yellow	Yellow	Greener	No change
(439)[715] *Cyanol Extra:*				
0.01	Yellow	Greenish yellow	Green	No change
0.002	Faint yellow	....	Decolor.	No change
(585)[842] *Methylene Violet 2 RA:*				
0.01	Pale blue	Pale green	Slightly fainter	No change
0.002	N. decolor.	Greenish yellow	Slightly fainter	No change

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)
A. Violet and Blue Colors

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(601)[861] *Induline Soluble:*				
0.01	Fainter	Poor violet	Violet	Blue violet
0.002	Fainter	Brownish	N. decolor.	Decolor.
(602)[865] *Nigrosine Soluble:*				
0.01	Violet	Dark blue	Brown	Brown
0.002	Violet	Blue	Brown	Brown
(650)[922] *Methylene Blue:*				
0.01	Blue green	Bright green	Paler	No change
0.002	N. decolor.	Green	N. decolor.	No change
(692)[1180] *Indigotine:*				
0.01	More bluish	Dark blue	Greenish yellow	No change
0.002	N. decolor.	Blue	Yellow	N. decolor.
Brilliant Blue FCF:				
0.01	Green to yellow	Blue green	No change	No change
0.002	Green to yellow	Blue green	No change	No change

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(53)[77] *Palatine Scarlet:*				
0.01	Crimson	Magenta	Yellow brown	No change
0.002	Pinker	Pink violet	Light brown	No change
(55)[79] *Xylidine Red:*				
0.01	Crimson	More crimson	Orange	No change
0.002	Pinker	Orange pink	Brown	No change
(56)[80] *Ponceau 3R:*				
0.01	Deeper pink	Scarlet	Red orange	No change
0.002	Deeper pink	Slightly more orange	Red orange	No change
(65)[88] *Bordeaux B:*				
0.01	Violet	Blue	Red brown	No change
0.002	More violet	Greenish blue	Almost decolor.	Slightly decolor.
(64)[89] *Crystal Ponceau:*				
0.01	Violet	Blue	Brown	No change
0.002	More violet	Blue	Brown	No change

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)
B. Reds

Color	Concentrated HCl	Concentrated H₂SO₄	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(71)[114] *Azoeosine:*				
0.01	Red violet	Dark red violet	Orange	Red orange
0.002	Faintly more violet	Dark brown	N. decolor.	Dirty salmon
(102)[176] *Fast Red A:*				
0.01	Violet red	Blue violet	Dark red	No change
0.002	Violet red	Poor violet	Orange pink	No change
(103)[179] *Azorubine S:*				
0.01	Deepens slightly	Blue violet	Orange red	Redder
0.002	Deepens slightly	Dirty violet	N. decolor.	Decolor.
(107)[184] *Amaranth:*				
0.01	Violet red	Violet	Red brown	Slightly decolor.
0.002	No change	Dirty violet	N. decolor.	Decolor.
(106)[185] *Brilliant Scarlet:*				
0.01	Crimson	Violet	Brown	No change
0.002	Pinker	Poor lavender	Yellow brown, turns paler	No change
(108)[186] *Ponceau 6R:*				
0.01	Crimson	Dark violet	Brown	No change
0.002	No change	Brownish violet	Yellow brown	No change
(160)[277] *Croceine Scarlet 3B:*				
0.01	Dark blue	Magenta	Poor violet brown	No change
0.002	Blue	Violet red	Brown	No change
(163)[280] *Biebrich Scarlet:*				
0.01	Dark blue	Dark green	Deep violet	No change
0.002	Blue	Green	Violet	Decolor., more yellow.
(169)[286] *Croceine Scarlet 7B:*				
0.01	Greenish blue	Greenish blue	More violet	No change
0.002	Light greenish blue	Dirty brownish blue	N. decolor., brown pink	No change
(240)[370] *Congo:*				
0.01	Dark blue	Very dark blue	More orange	More orange
0.002	Blue	Dirty blue	More orange	More orange

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)
B. Reds

Color	Concentrated HCl	Concentrated H₂SO₄	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(277)[448] *Benzopurpurine 4B:*				
0.01	Greenish blue	Dark blue	More orange	More orange
0.002	Blue	Blue	Orange pink	No change
(278)[449] *Benzopurpurine 6B:*				
0.01	Blue	Dark blue	More orange	More orange
0.002	Bluish green	Bluish green	More orange	More orange
(448)[677] *Magenta:*				
0.01	Yellow brown	Brown	N. decolor.	N. decolor.
0.002	Decolor.	Pale brown	Decolor.	Decolor.
(462)[692] *Acid Magenta:*				
0.01	N. decolor.	Brownish yellow	Decolor.	Decolor.
0.002	N. decolor.	Brownish yellow	Decolor.	Decolor.
(504)[749] *Rhodamine B:*				
0.01	Pink orange	Poor yellow	Bluer	No change
0.002	Decolor.	Poor yellow	N. decolor.	No change
(512)[768] *Eosine:*				
0.01	Orange	Orange	No change	No change
0.002	Yellow	Yellow	No change	No change
(517)[773] *Erythrosine*				
0.01	Deep orange	Orange	No change	No change
0.002	Orange yellow	Orange	No change	No change
(518)[774] *Phloxine P:*				
0.01	Yellow	Deep orange yellow	No change	Pink
0.002	Pale yellow	Yellow orange	No change	Pink
(520)[777] *Rose Bengal:*				
0.01	Yellow	Orange	Slightly deeper	No change
0.002	Decolor.	Light brown	No change	No change
(523)[779] *Rose Bengal 3B:*				
0.01	Red orange	Red orange	No change	No change
0.002	Decolor.	Yellow	No change	No change
(584)[841] *Safranine:*				
0.01	Greenish blue	Green	Slightly redder	No change
0.002	Decolor.	Light brown	Decolor.	No change

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)

B. Reds

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(710)[1242] *Archil:*				
0.01	Scarlet	Brown	Violet	Violet
0.002	Pink	Light brown	Violet	Violet
Ponceau SX:				
0.01	Red	Brown	Orange	Orange
0.002	Red	Brown	Orange	Orange

C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(1)[7] *Picric Acid:*				
0.01	Decolor.	N. decolor.	Orange	No change
0.002	Decolor.	N. decolor.	Orange	No change
(3)[9] *Martius Yellow:*				
0.01	Decolor.	Paler	No change	No change
0.002	Decolor.	Paler	No change	No change
(4)[10] *Naphthol Yellow S:*				
0.01	Decolor.	N. decolor.	Paler	Paler
0.002	Decolor.	N. decolor.	Paler	Paler
(8)[16] *Fast Yellow B:*				
0.01	Red	Darker	No change	No change
0.002	Pink	Darker	No change	No change
(13)[26] *Croceine Orange*				
0.01	Orange red	Deeper orange	Brownish orange	No change
0.002	Pink	Deeper orange	N. decolor.	No change
(14)[27] *Orange G:*				
0.01	Scarlet	Crimson	Brownish orange	No change
0.002	Pink	Brownish pink	Brown	No change
(48)[63] *Orange GT:*				
0.01	Red	Darker	Brownish orange	No change
0.002	Pink	Darker	Partly decolor.	No change
(95)[138] *Metanil Yellow:*				
0.01	Red violet	Black violet	No change	No change
0.002	Red violet	Blue violet	No change	No change
(87)[142] *Orange III:*				
0.01	Violet red	Blue violet	No change	No change
0.002	Violet red	Blue violet	No change	No change

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Continued*)
C. Yellows and Oranges

Color	Concentrated HCl	Concentrated H₂SO₄	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(88)[143] *Orange IV:*				
0.01	Dark red violet	Deep blue violet	Deeper	No change
0.002	Violet	Blue violet	Deeper	No change
(89)[144] *Brilliant Yellow S:*				
0.01	Red violet	Magenta	No change	No change
0.002	Red violet	Magenta	No change	No change
(84)[148] *Resorcin Yellow:*				
0.01	Deep orange	Orange	Orange red	Slightly yellower
0.002	Orange	More yellow	Poor orange red	Slightly yellower
(85)[150] *Orange I:*				
0.01	Deep violet	Blue violet	Deep violet red	Deep red
0.002	Violet	Violet	Red	Pink
(86)[151] *Orange II:*				
0.01	Crimson	Red violet	Orange red	No change
0.002	Pink	Violet	N. decolor.	No change
(269)[480] *Chrysamine R:*				
0.01	Red violet	Blue violet	Red orange	No change
0.002	Red violet	Blue violet	Red orange	No change
(94)[640] *Tartrazine:*				
0.01	No change	No change	No change	No change
0.002	No change	No change	No change	No change
(425)[655] *Auramine:*				
0.01	Decolor.	N. decolor.	Decolor.	Paler
0.005	Decolor.	N. decolor.	Decolor.	Paler
(510)[766] *Uranine:*				
0.01	More greenish	Deeper	Orange	Orange
0.005	More greenish	Deeper	Orange	Orange
(667)[801] *Quinoline Yellow:*				
0.01	Darker	Brownish yellow	N. decolor.	No change
0.002	Darker	Brownish yellow	Decolor.	No change
Sunset Yellow FCF:				
0.01	Orange red	Orange red	Red brown	No change
0.002	Red orange	Red orange	Yellowish brown	No change

TABLE 5.—COLOR REACTIONS OF DYED FIBER.—(*Concluded*)
D. Greens

Color	Concentrated HCl	Concentrated H_2SO_4	10 per cent NaOH	Ammonia (sp. gr. 0.96)
(398)[5] *Naphthol Green B:*				
0.05	Yellow	Brownish yellow	No change	No change
0.01	Decolor.	Brownish yellow	No change	No change
(427)[657] *Malachite Green:*				
0.01	Orange	Green to orange	Decolor.	Decolor.
0.002	Orange	N. decolor.	Decolor.	Decolor.
(428)[662] *Brilliant Green:*				
0.01	Orange	Green to orange	Decolor.	Decolor.
0.002	Decolor.	N. decolor.	Decolor.	Decolor.
(433)[666] *Guinea Green B:*				
0.01	Orange	Yellow brown	Orange	Decolor.
0.002	Orange	Yellow	Orange	Decolor.
(435)[670] *Light Green SF Yellowish:*				
0.01	Orange	Orange	Decolor.	Decolor.
0.002	N. decolor.	Orange	Decolor.	Decolor.
(602)[865] *Nigrosine Soluble:*				
0.01	Violet	Dark blue	Brown	Brown
0.002	Violet	Blue	Brown	Brown
Fast Green FCF:				
0.01	Orange brown	Green then yellow	Blue	Blue
0.002	Orange	Green then yellow	Blue	Blue

E. Oil-soluble Colors
(Dyed on silk)

(7)[15] *Aniline yellow*	Brown	Brown	No change	No change
(16)[19] *Butter yellow*	Red	Yellow	Yellow	No change
[22] *Yellow AB*	Violet red	Violet	No change	No change
(10)[23] *Sudan G*	Brown	Brown	Brown	No change
(11)[24] *Sudan I*	Orange red	Red	No change	No change
[61] *Yellow OB*	Red	Blue violet	No change	No change
(143)[248] *Sudan III*	Violet	Blue violet	Darker	No change
FD&C No. 2 *Orange SS*	Red violet	Red	No change	No change
FD&C No. 32 *Oil Red XO*	Blue violet	Red violet	No change	No change

TABLE 6.—REACTIONS OF DYES IN AQUEOUS SOLUTION AND WITH
CONCENTRATED SULPHURIC ACID

No.	HCl	NaOH	Color of dry dye	Dry color + sulphuric acid	
				Before dilution	After dilution
Blues and Violets:					
(287)[463]	No change	Crimson	Violet	Blue	Violet
(451)[680]	Greenish	No change	Purple	Orange	Yellow green, then greenish blue
(452)[681]	Green	No change	Purple	Orange yellow	Yellow green then greenish blue
(464)[695]	Blue green to yellow	No change	Purple	Orange	Yellow green, then greenish blue
(468)[698]	Green	No change	Violet	Orange	Yellow
(476)[703]	No change	Pinker	Blue	Red	Blue (and blue ppt.)
(480)[707]	No change	Redder	Blue violet	Red brown	Blue
(440)[712]	Yellow green	No change	Greenish blue	Pale yellow nearly colorless	Dark yellow
(439)[715]	Green	More bluish	Blue	Pale yellow	Deeper yellow
(585)[842]	Bluer	Redder	Red violet	Green	Red violet
(601)[861]	Bluer	Redder	Violet blue	Blue violet	Violet blue
(602)[865]	Violet	Slightly redder	Gray black	Blue	Violet
(650)[922]	No change	No change	Blue violet	Green	Blue
(692)[1180]	No change	Bright green	Blue	Violet blue	Blue
Brilliant Blue FCF	Blue green	No change	Blue	Pale yellow	Yellow green
Reds:					
(53)[77]	No change	More orange	Red	Magenta	Rose pink
(55)[79]	No change	Deeper, then decolorized	Red	Crimson	Red
(56)[80]	Pinker	Yellower, decolorized with weaker solution	Red	Crimson	Rose pink
(65)[88]	No change	Orange, dilute sol. decolor.	Violet	Blue	Magenta
(64)[89]	No change	Orange, dilute sol. decolor.	Red violet	Blue	Magenta
(71)[114]	No change	Orange	Red	Magenta	Pink
(102)[176]	Yellow brown	No change	Red	Blue violet	Red orange
(103)[179]	No change	More orange	Red violet	Blue violet	Red
(107)[184]	No change	Less pink	Red violet	Blue violet	Red
(106)[185]	No change	Red brown	Red	Red violet	Crimson
(108)[186]	No change	Red brown	Brown	Blue violet	Crimson
(160)[277]	No change	Violet brown	Red	Red violet	Brown ppt., then red ppt.
(163)[280]	No change	Purple	Red	Bluish green	Red
(169)[286]	No change	More violet	Violet red	Blue	Crimson
(240)[370]	Blue	Orange	Red	Blue	Blue ppt.
(277)[448]	Purple	More orange	Red	Blue	Blue ppt.
(278)[449]	Violet	More orange	Red	Blue	Blue
(448)[677]	Yellow	Decolorized	Purple	Orange	Yellow
(462)[692]	No change	Decolorized	Violet	Orange	Magenta
(504)[749]	No change	No change	Violet	Yellow	Pink
(512)[768]	Yellow fluor. destroyed	No change	Magenta	Yellow	Orange red
(517)[773]	Yellow, then decolor.	No change	Violet	Orange	Pink
(518)[774]	Decolorized	No change	Crimson	Orange	Colorless
(520)[777]	Decolorized	No change	Violet	Orange	Faint pink
(523)[779]	Decolorized	No change	Violet	Orange	Pale pink
(584)[841]	More violet	No change	Violet red	Blue	Crimson
Ponceau SX.	No change	More orange	Red	Crimson	Red

TABLE 6.—REACTIONS OF DYES IN AQUEOUS SOLUTION AND WITH CONCENTRATED SULPHURIC ACID.—(*Concluded*)

No.	HCl	NaOH	Color of dry dye	Dry color + sulphuric acid	
				Before dilution	After dilution
Yellows and Oranges:					
(1)[7]	No change	No change	Yellow	Colorless	Yellow
(3)[9]	Decolorized	No change	Red orange	Yellow	Pale yellow
(4)[10]	Decolorized	No change	Orange	Yellow	Yellow
(8)[16]	Pink orange	No change	Brownish	Orange	Red
(13)[26]	No change	Redder	Orange red	Orange	Orange
(14)[27]	No change	Pinker	Red	Crimson	Orange red
(43)[63]	No change	No change	Orange red	Orange red	Orange
(95)[138]	Magenta	No change	Brown	Blue violet	Red violet
(87)[142]	Pinker	No change	Brown yellow	Blue violet	Red violet
(88)[143]	Crimson	No change	Orange	Blue violet	Red violet
(89)[144]	Darker	No change	Yellow	Red violet	Yellow brown
(84)[148]	No change	Redder	Orange red	Orange	Orange
(85)[150]	Darker	Brown red	Red brown	Blue violet	Magenta, then orange
(86)[151]	No change	Reddish brown	Orange red	Red violet	Orange
(269)[480]	Decolorized	Red orange	Orange	Blue violet	Pale yellow
(94)[640]	No change	No change	Orange	Yellow	Yellow
(425)[655]	No change	Decolorized	Yellow	Colorless	Yellow
(510)[766]	Yellow fluor. destroyed	No change	Orange red	Yellow	Yellow
(667)[801]	No change	No change	Yellow	Orange	Yellow
Sunset Yellow FCF	No change	Redder	Red orange	Red orange	Orange yellow
Greens:					
(398)[5]	Paler	No change	Green	Orange	Yellow
(427)[657]	Green, then orange	Decolorized	Blue green	Yellow	Red orange—green
(428)[662]	Green, then orange	Decolorized	Green	Yellow	Red orange
(433)[666]	Yellowish green	Decolorized	Blue green	Yellow	Green
(435)[670]	Yellowish green	Decolorized	Green	Orange	Greenish blue
Fast Green FCF	Yellowish green	Purple	Blue green	Yellow	Greenish blue

Oil-soluble Colors
(Tests with HCl and NaOH on Alcoholic Solution)

No.	HCl	NaOH	Color of dry dye	Before dilution	After dilution
(7)[15]	Orange	No change	Yellow	Yellow	Orange
(16)[19]	Red	No change	Yellow	Greenish yellow	Red
[22]	Redder	No change	Yellow	Blue violet	Orange
(10)[23]	No change	Redder	Orange	Yellow orange	Yellow
(11)[24]	No change	Red	Orange red	Red	Orange
[61]	Redder	No change	Orange	Blue violet	Orange
(143)[248]	No change	Redder	Red	Blue violet	Pink
FD&C Orange No. 2	No change	Redder	Orange red	Red	Orange
FD&C Red No. 32	No change	Redder	Red	Red	Pink

The reactions given in Table 6 on page 88, if they have not been used in the analytical scheme, should also be tried. (See directions for making the tests on page 75.)

An excellent plan, and one always to be followed in doubtful cases, is to dye a piece of wool, silk in the case of the oil-soluble colors, with a genuine sample of each of the doubtful colors, taking care to have the intensity of the dyed fabric about equal that of the sample dyed from the food product, and then to apply the reagents in directly comparable tests to all the samples.

Collections of samples of the colors likely to be met in foods may be obtained from Eimer and Amend, New York.

Separation of Mixtures.—Sometimes the color present is not a single dye, but a mixture of two or more is used to produce the shade desired, as green from a mixture of blue and yellow or red and yellow to produce orange. Some of the more common combinations are shown in the following table:[1]

Egg shade: Tartrazine + Orange I

Greens:	Tartrazine + Indigotine	(pistachio)
	Tartrazine + Brilliant Blue FCF	(lemon)
	Tartrazine + Fast Green FCF	(lemon)
	Tartrazine + Guinea Green B	(lemon)
	Tartrazine + Light Green SF Yellowish	(lemon)

Orange:	Tartrazine + Orange I	
	Tartrazine + Ponceau 3R	
	Tartrazine + Sunset Yellow FCF	
	Tartrazine + Ponceau 3R + Amaranth	

| *Brown:* | Ponceau 3R + Orange I | (chocolate and frankfurters) |

Violet:	Amaranth + Indigotine	(grape color in jams,
	Amaranth + Brilliant Blue FCF	jelly, etc.)
	Amaranth + Fast Green FCF	
	Amaranth + Ponceau 3R + Orange I	

The presence of such a mixture may usually be determined in dyeing on wool if the fractional dyeing as described on page 71 be employed. In the case of a mixture there will generally be a gradual change in the color of the wool from the first dye-

ings to the last. By combining the end fractions, stripping and redyeing, enough color can frequently be separated for identification.

A better method, however, is by extraction with immiscible solvents, being an elaboration of the method described on page 73. After separating the basic from the acid dyes by shaking out the former with amyl alcohol or ether from an alkaline solution, most of the acid colors can be extracted by amyl alcohol from the strongly acidified solution. If now the amyl alcohol be washed with successive portions of water, the dyes are removed at different stages, depending in general upon the degree to which they are sulphonated. Still others are removed from the amyl alcohol only by treatment with petroleum ether or by shaking out with dilute alkali.

Such a procedure, based very largely upon work done in the New York Food Inspection Laboratory,[1] is given below for the coal-tar dyes included in the table on page 67. A more elaborate scheme, covering a greater number of dyes, is given by Mathewson,[2] and procedures for the separation of the various permitted dyes when present together have been devised by Price[3] and others for mixtures of the dry colors.

In carrying out the extractions, it should be remembered that the degree of acidity and the consequent separation of the colors depend largely upon the comparative volumes used and the time of shaking; hence when these points are specified they should be closely followed. The solution to be tested should also be as free as possible from extraneous matter.

Procedure. *a. Separation of Basic Colors.*—Make a small portion of the color solution quite strongly alkaline with sodium hydroxide and shake with ether. Separate the ether and shake it with dilute acetic acid. If the ether is colored or yields a color to the acetic acid a basic color is probably present. If no color is noticed, make the acetic acid solution alkaline with ammonia and evaporate to dryness on the water bath. A basic dye that imparts no color to the ether or the acetic acid may usually be detected in this way.

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[1] MATHEWSON: U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, p. 53.

[2] MATHEWSON: U. S. Dept. Agr., *Bull.* **448**, p. 18.

[3] ESTES: *Ind. Eng. Chem.*, **1916**, 1123; INGERSOLL: *ibid.*, **1917**, 955; PRICE: U. S. Dept. Agr., *Bur. Animal Industry Circ.* **180**.

If a basic color is found, add to the remainder of the original color solution one-fifth its volume of 10 per cent sodium hydroxide and extract three times with half its volume of ether. *Avoid violent shaking and the consequent formation of troublesome emulsions.* Shake the combined ether extracts three times with half the volume of water. Preserve the first two extracts and reject the third. Finally, shake the ether twice with half its volume of dilute acetic acid (one part of acid, sp. gr. 1.04, to two of water). For the particular dyes that will be found at various points in this procedure consult the Outline, page 93, remembering that to show the presence of the dye it may be necessary to evaporate the solution on the water bath as described above.

b. Separation of Acid Colors.—Add to 25 or 50 cc. of the original color solution one-half its volume of concentrated hydrochloric acid. If basic colors have been previously extracted the solution should be neutralized with hydrochloric acid before adding the excess of strong acid. Shake three times with amyl alcohol and combine the extracts, which should have a total volume not greater than that of the original solution used. Shake the amyl alcohol with successive portions of water of about one-half its volume until the last portions are perfectly neutral. Seven of these washings should be obtained and preserved for further examination. If color still remains in the amyl alcohol, dilute it with two volumes of gasoline or petroleum ether and shake out twice with water as before. Separate further any colors obtained at this point as described in Sec. 5 of the Outline.

Finally shake the amyl alcohol-petroleum ether mixture twice with 2 per cent sodium hydroxide solution.

The original solution, from which basic dyes have been removed with ether and acid dyes with amyl alcohol, although perhaps perfectly colorless, may still contain a few colors, including several permitted dyes. Make the solution slightly alkaline with ammonia, acidify slightly with acetic acid and shake out twice with amyl alcohol. In case it is desired to separate still further the few dyes still remaining in the aqueous solution it may be extracted with small quantities of dichlorhydrin. This, however, is a rather expensive reagent and in the majority of cases will not be necessary. The separation is summarized in Group *C* of the Outline.

Notes.—If any of the vegetable colors which are considered later in this chapter are present, they will be extracted by the amyl alcohol from the strongly acid solution and appear at various points in the washing out with water. Logwood and saffron will be found mainly in fractions 1 and 2 and the petroleum ether fraction; cochineal will appear in Sec. 4 of the Outline; annatto, archil, Persian berries, and turmeric will remain in the amyl alcohol even after the treatment with petroleum ether but they are removed by the washing with dilute (2 per cent) sodium hydroxide.

This method of separation depends upon the degree of sulphonation of the dyes and their consequent solubility. On washing the amyl alcohol solution with water, in general the higher sulphonated dyes come out first, while the wash water still contains much hydrochloric acid; the lower sulphonated ones later; and finally the unsulphonated acid colors as Erythrosine, Martius Yellow, etc. If a sufficient separation has not been thus effected, the fractions containing the chief amounts of each color may be united and put through the necessary part of the procedure again.

Outline

The following tabulation shows the points at which the various dyes will appear in the procedure. It must be remembered, however, that a given dye will usually appear in several washings and the table only indicates where the *maximum* amount will be found. The numbers of the dyes refer to the list on page 67. In order to avoid confusion where the numbers follow so closely, only those of the "Colour Index" are given.

A. *Basic Dyes.*—Extracted by ether from strongly alkaline solutions. (922 extracted only in small amount, perhaps with decomposition.)
   1. Readily removed from ether on washing with water: 677, 841, 842.
   2. More or less slowly removed by water, quickly by acetic acid: 655, 657, 662, 680, 681, 749.

B. *Acid Dyes.*—Not extracted by ether. Extracted by amyl alcohol from the strongly acidified solution.
   1. Removed in first washings of amyl alcohol, acidity high. (Mostly in fractions 1 and 2): 16, 144, 186, 463, 1180, Brilliant Blue FCF.
   2. Removed at lower acidity, but usually above $0.25N$.
   (Mostly in fractions 2 and 3): 184, 185, 640, 865, Sunset Yellow FCF.

3. Removed at rather low acidity.   (Mostly in fractions 3, 4 and 5):
   27, 77, 89, 707.

4. Removed at very low acidity, but before washings are neutral.
   (Mostly in fractions 4, 5, and 6): Combine the most deeply
   colored fractions, add one-half volume of HCl (1.12) and shake
   out with amyl acetate.

   a. Readily extracted: 7, 10, 277, 280, 286, (449).

   b. Not readily extracted: 79, 80, 88, 148, 179, Ponceau SX,
       Fast Green FCF.

5. Removed by water from the practically neutral solvent, most
   readily after the addition of petroleum ether.   (Fractions 6, 7,
   and the petroleum ether fractions.)   Combine the most deeply
   colored fractions, add one-fifth volume of HCl (1.20) and shake
   out with an equal volume of ether.

   a. Almost no color extracted.   Add 5 per cent of NaCl and shake
       with amyl alcohol.   Separate the amyl alcohol and shake it
       with 5 per cent Na_2CO_3 solution.

       (1) Almost completely extracted: 150.

       (2) Not readily extracted: 138, 142, 143, 801.

   b. Almost all extracted: 26, 63, 114, (142), 151, 176, 448, (449).

6. Removed by dilute sodium hydroxide: 9, 480, 766, 768, 773, 774,
   777, 779.

C. Not extracted by ether.   Not extracted from the strongly acidified
   solution by amyl alcohol.   (5 is decomposed; 865 separates as a
   precipitate but is extracted by dichlorhydrin.)

   Add NH_4OH until slightly alkaline, then acetic acid until slightly
   acid, and shake out with amyl alcohol.

   a. Readily extracted: 666, 695, 698, (922).

   b. Not readily extracted.   Shake with small quantities of
       dichlorhydrin.

       (1) Readily extracted: 370, 670, 712, 715, Brilliant Blue FCF,
           Fast Green FCF.

       (2) Not readily extracted: 692.

If this simple scheme is not sufficient, or for a more thorough
separation and identification of the permitted colors only,
including the oil-soluble dyes, consult the "Official Methods" of
the Association of Official Agricultural Chemists, **1935,** pages
236 to 244.

Vegetable Colors.—The colors of animal or vegetable origin
which are most likely to be found in foods are comparatively few,
the use of such colors having greatly decreased owing to the
advantages of the coal-tar dyes.   The following are those of

chief importance: Annatto, archil, caramel, chlorophyll, cochineal, cudbear, logwood, Persian berries, saffron, and turmeric.[1]

Detection of Vegetable Colors.—All these colors, except caramel and chlorophyll, may be extracted from a solution or suspension of the food material by acidifying with hydrochloric acid and shaking out in a separatory funnel with amyl alcohol. The extract obtained in this way, however, may contain fat, sugars, and other portions of the food which would interfere with subsequent tests for the color, so it must be carefully purified. To do this, wash the amyl alcohol layer twice with small quantities of water, and evaporate the amyl alcohol on the water bath. Take up the residue in 50 per cent alcohol, filter, and shake twice with petroleum ether to remove fat. Draw off the alcohol layer, dilute with an equal volume of water, add a few drops of hydrochloric acid, and shake out again with amyl alcohol. This amyl alcohol may be washed once with water to remove the excess of acid and the fairly pure color obtained by evaporation on the water bath.

To identify the color, it may be dissolved in water and tested as in Table 7.[2] The reduction test with zinc and hydrochloric acid is conveniently made in a test tube, using about 0.2 gram of zinc dust and 10 drops of strong acid. The test with sulphuric acid on the dry color may be made on small amounts of color by streaking the acid with a glass rod across a spot of the evaporated solution in a porcelain dish. The reactions should be observed in as concentrated a solution of the color as can be obtained.

A portion of the solution should also be acidified with a drop of dilute hydrochloric acid and evaporated nearly to dryness with a piece of wool previously mordanted with tin.[3] The dyed wool is washed and dried, then portions of it tested with reagents as in Table 8, page 97.[4]

If necessary, the special tests described below may be used in confirmation. It should be remembered that many of the natural fruit colors dissolve in amyl alcohol, giving a colored

[1] A long list of colors is given by Seeker in Allen's "Commercial Organic Analysis," 4th ed., Vol. V.

[2] Loomis: U. S. Dept. Agr., *Bur. Chem. Circ.* **63.**

[3] In 500 cc. of water dissolve 0.8 gram of tin crystals (SnCl₂) and 0.4 gram of oxalic acid. Boil 10 grams of fat-free wool 1½ hours in this solution. Wring and dry at room temperature. Keep in a stoppered bottle away from the light.

[4] Berry: U. S. Dept. Agr., *Bur. Chem. Circ.* **25.**

TABLE 7.—REACTIONS OF COLORS IN AQUEOUS SOLUTION AND WITH CONCENTRATED SULPHURIC ACID

Name of color	Color of aqueous solution	Add to aqueous solution				Dry color + conc. H_2SO_4	
		HCl (1.12) 5–10 drops	10 per cent. NaOH 5–10 drops	NH₄OH (0.96) 5–10 drops	Zn dust + HCl and exposure to air on filter paper	Before dilution	After dilution
Annatto	Yellow in alkaline solution	Paler	....	....	....	Blue	Mauve
Archil (Cudbear)	Deep lilac	Yellowish pink	Purple	Purple	Orange; restored	Purple	Red to orange
Cochineal	Orange red	Orange yellow	Magenta	Magenta	Orange yellow; not restored	Pink	Yellow pink then straw yellow
Logwood	Yellowish brown	Orange	Dark brown	Light brown	Color not restored	Yellow brown	Paler
Persian berries	Yellow	No change	Orange	Deeper	Not decolorized	Yellow	Paler
Saffron	Yellow	No change	Paler	No change	Color not restored	Blue, purple, maroon, red brown	Yellow, then nearly colorless
Turmeric	Yellow in alkaline solution	Paler	....	....	With Zn and NaOH not decolorized	Orange	Dirty yellow

TABLE 8.—REACTIONS OF VEGETABLE COLORS ON WOOL, MORDANTED WITH TIN

Color	Hydrochloric acid (sp. gr. 1.16)	Sulphuric acid (sp. gr. 1.84)	Nitric acid	Stannous chloride, hydrochloric acid and water	10 per cent sodium hydroxide	Boiling alcohol
Annatto	Unchanged	Olive green	Lemon yellow	Unchanged	Fiber and solution bluish purple; color slowly removed	Bluish red solution
Archil and Cudbear	Solution and fiber red	Fiber and solution purple; on dilution red, fiber almost colorless	Yellow	Decolorized	Orange	....
Cochineal	Orange red	Dark violet	Yellow	Orange	Red violet	No color
Logwood	Red violet	Olive brown, yellow on dilution	....	Red to violet	Violet	No color
Persian berries	Little affected	Yellow olive	....	Fiber yellower	....	....
Saffron	Darker	Olive green, then red brown; fades on dilution	....	Not decolorized	Darker	....
Turmeric	Fiber reddish, solution pale pink; on dilution fiber bright yellow	Fiber and solution reddish brown; on dilution fiber pale straw, solution colorless	At first deep red, then yellow	Bright reddish orange fiber and solution	....	Yellow solution, green fluorescence.

solution, and the analyst should be assured that the reactions obtained are not due to the fruit color.[1] It is obvious, also, that the absence of coal-tar dyes must be assured before making the tests.

It is always best to confirm any conclusions by tests made on an actual sample of the color in question.

Special Tests for Vegetable Colors. *Archil and Cudbear.*— These two lichen colors dye wool in the same manner as the coal-tar dyes and hence are included in that section (pages 70 to 93). They differ markedly from the other vegetable colors in dyeing, stripping, and redyeing wool readily.

Caramel.—Practically the only positive test for small amounts of caramel is the precipitate that it gives with paraldehyde.[2] The test is carried out by adding to the clear color solution, concentrated to small bulk, three to five times its volume of paraldehyde, then adding alcohol in small portions with frequent shaking until the mixture is just homogeneous. Avoid excess. If caramel is present, a brown adherent precipitate will form on standing (overnight in the case of small amounts).

To use this test successfully, several precautions must be observed. Care should be taken in concentrating the solution of color that the heat employed is not sufficient to caramelize any sugar that may be present. The temperature should not rise above 70°C. or so. In adding the alcohol, only enough should be added so that the aqueous solution and the paraldehyde do not separate on standing for several minutes, not necessarily enough to make the mixture entirely free from turbidity. If considerable caramel is present, the liquid will be turbid and precipitation may commence as soon as the liquids have been mixed. In the case of materials containing much sugar or gum, paraldehyde may cause a precipitate resembling caramel. In such cases it is advisable to separate the caramel by a preliminary precipitation, conveniently with zinc hydroxide, which exercises a somewhat selective action, dragging down the caramel to a greater extent than the gum and sugar.[3]

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[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **107** (rev.), p. 193, and *J. pharm. chim.*, **1901**, 174.

[2] AMTHOR: *Z. anal. Chem.*, **1885**, 30.

[3] WOODMAN and NEWHALL: *Tech. Quart.*, **1908**, 280; *J. Assoc. Off. Agr. Chem.*, **1935**, 247.

To 10 to 20 cc. of the practically neutral solution to be tested, add 2 cc. of zinc chloride (5 per cent solution) and 2 cc. of potassium hydroxide (2 per cent solution). This is done preferably in a small test tube. Stir thoroughly and centrifuge. Pour off the liquid, add 25 cc. of boiling water to the precipitate, mix, centrifuge and again pour off the liquid. Repeat, if necessary, until the wash water is colorless. Dissolve the precipitate in 15 cc. of 10 per cent acetic acid. Concentrate the acid solution to about one-third of its volume, carefully neutralize the excess of acid, filter the solution if not perfectly clear, and precipitate with paraldehyde as above.

Other tests are sometimes employed to show the presence of caramel in foods, but they are for the most part negative tests, depending on analytical differences between the caramel and the natural color of the particular food and are not of general application. Such tests, based on the insolubility of caramel in ether, chloroform, or amyl alcohol, are described under Vanilla and Whisky, pages 462 and 575.

Cochineal.—If a portion of the amyl alcohol solution of the color is shaken with dilute ammonia, a purple color is produced in the presence of cochineal. A still more characteristic test is given with uranyl acetate. To the amyl alcohol solution add a little sodium acetate (the test with uranyl acetate is not satisfactory with a large excess of free hydrochloric acid), shake with water, and add a 3 per cent solution of uranyl acetate drop by drop, with thorough shaking after each drop. In the presence of cochineal a characteristic emerald-green color is produced.

Saffron.—Saffron is most likely to be encountered in such products as macaroni, noodles, and pastry. The best solvent for extracting it is 70 per cent alcohol made slightly ammoniacal (3 drops of 0.96 ammonia to 100 cc. of 70 per cent alcohol). The coarsely ground material is digested at room temperature for several hours with the solvent, which is then filtered off. The acid coal-tar dyes, like Naphthol Yellow S, that are added to macaroni are also readily dissolved by this treatment and can be dyed on wool and identified as directed under coal-tar colors.

The 70 per cent alcohol extracts also the "lutein" or yellow coloring matter of the flour, as well as color from the eggs if any are present. These can be removed, however, by evaporat-

ing the alcoholic solution to dryness on the water bath and extracting the residue with ether, in which the saffron and most of the coal-tar colors that are used are nearly insoluble, while the natural color dissolves. If preferred, the original material may be extracted with ether before removing the artificial color with the 70 per cent alcohol. A mixture of 7½ parts of acetone to 1 of water may also be used for the preliminary extraction. The saffron may be taken out from the aqueous solution left after evaporating the alcohol by acidifying with hydrochloric acid and extracting with amyl alcohol. It is best to purify the color as described on page 95, after which the purified residue may be tested for saffron by drawing across it a glass rod moistened with strong sulphuric acid, which gives an immediate but very fugitive blue color. A similar color is given with concentrated nitric acid. It is essential that the color be as pure as possible, since otherwise the charring with the acid will produce red or purple colors which mask the saffron reaction. The blue color of the saffron test appears *immediately*, changing quickly to red and finally to brown.

Turmeric.—This is readily detected by the well-known boric acid test. An alcoholic extract of the material is evaporated almost to dryness on the water bath with a piece of filter paper and a few drops of a saturated solution of boric acid. If turmeric is present, the dried paper will be a cherry-red color, which is changed to bluish green by a drop of sodium hydroxide or ammonia.

Mineral Colors.—The class of mineral colors may include pigments used directly, as oxide of iron (red ochre) in coloring anchovy and similar fish pastes, and ultramarine for coloring sugar; or mineral compounds of colors, as the so-called "lakes." These are insoluble compounds of vegetable colors or of coal-tar dyes, usually with a metallic base, as aluminum or tin.

The mineral colors, being insoluble, are usually readily seen as colored particles when a portion of the food material is examined under the microscope, and their presence may be confirmed by testing the ash for such metals as iron, tin, aluminum, lead, chromium, or antimony. Minute traces of iron or aluminum should be ignored, since they occur naturally in the ash of many food materials. Some of the lakes, especially of cochineal and logwood, are used quite extensively in confectionery; hence if

added color is suspected and the ordinary tests for coal-tar dyes or vegetable color are negative, the possibility of a lake being present should be considered. In such cases, the lake may be decomposed with hydrochloric acid and the color extracted by amyl alcohol and tested as described under the other classes of colors. It is often helpful to shake the material with water, collect the insoluble pigment or lake from the washings by means of a centrifuge, and examine the sediment for the identification of the color.

CHEMICAL PRESERVATIVES

General.—From ancient times certain methods and materials have been used in the treatment of foods in order to prevent fermentation and decay, and from an economic standpoint such preservation is necessary in order to conserve for future use such food as cannot be consumed at the time or place where produced in the greatest abundance. So far as the consumer is concerned, however, an important feature in regard to the use of these older methods, such as smoking, salting, or pickling, is that the use of the preservative is at once evident to the senses, being recognized by the taste or odor. Such materials as sodium benzoate, salicylic acid, and the like, on the other hand, when present in food in amounts sufficient for preservation, being tasteless and odorless, are not thus evident to the consumer, who must rely upon the label or upon the result of chemical analysis. The detection of preservatives in food has been a matter of great importance, although their use has decreased much in recent years owing to strict enforcement of food legislation.

The most common of the "chemical" preservatives that have found use in food extensively in the past and are found at times even now are *formaldehyde, formic acid, salicylic acid, sodium benzoate, sulphurous acid* and *sulphites, borax* and *boric acid*, and *fluorides*. Saccharin, although commonly employed as a sweetening agent, has a distinct inhibitory effect on bacterial action and may properly be included. The various individuals vary with particular foods, as formaldehyde and borax in milk; salicylic acid in fruit juices; sodium benzoate in ketchup, jams, and sauces, sulphites in dried fruits, gelatin, and chopped meat; and fluorides in beer. Some of them, as borax, salicylic and benzoic

acids, and fluorides, occur naturally in notable amounts in certain food products, so that their mere presence is not always enough to show that a preservative has been *added*.

For a full discussion of the harmfulness of food preservatives or their desirability in foods, reference must be made to larger works and to official publications. Some of the principal arguments urged by those who would allow their use are: That they are used in such minute amounts as to be in no way harmful; that any possible danger from their use would be far less than from the use of foods partially spoiled on account of not having preservatives added; that foods in which these same chemical substances occur naturally are used with no injury resulting.

Those opposed to the use of preservatives urge: That their use is unnecessary, since if some manufacturers can prepare food without preservatives, all can; that their use is a constant temptation for unscrupulous manufacturers to put on the market unsuitable food, already partly decayed, but the fermentation arrested by the help of preservatives; that a preservative capable of arresting fermentation or bacterial action must have some degree of unwholesome effect on the process of digestion, since this is largely due to the action of enzymes and bacteria.

FORMALDEHYDE

Formaldehyde is a gaseous product of the partial oxidation of methyl alcohol, and is used as a food preservative as a dilute solution (2 to 30 per cent) in water. In the case of some food products, as milk, tests may be applied directly; with others, where interfering substances may be present, a portion should be acidified with phosphoric acid, distilled, and the first portion of the distillate tested by one of the methods given below.[1]

1. Hydrochloric Acid Test.[2]—Mix 5 cc. of the liquid to be tested with 5 cc. of pure milk in a small casserole, add 10 cc. of concentrated hydrochloric acid (containing 0.2 gram of ferric chloride per liter) and heat slowly nearly to boiling, occasionally giving the casserole a rotary movement to assist in dissolving the

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[1] For a critical study of many of the reactions for the detection of formaldehyde see GETTLER: *J. Biol. Chem.*, **1920**, 311.

[2] LEACH: *Mass. State Bd. Health, Ann. Rept.*, **1897**, 558; SHERMAN: *School of Mines Quart.*, **1905**, 408.

curd. Keep just below the boiling point for 1 minute, add 50 to 75 cc. of water and note the color carefully. In the presence of formaldehyde a violet color is produced, showing best at the moment of dilution.

Note.—The reaction that occurs in this test is a more or less general one for proteins, depending upon the presence of tryptophane in the protein molecule.[1] The same reaction is noticed when adding the sulphuric acid in making the Babcock test for fat in milk (page 131). The use of hydrochloric acid, by eliminating the charring effect of the sulphuric acid, renders the test more delicate. Even greater delicacy may be obtained by using bromine as the oxidizing agent.[2]

The test may also be modified by adding to 5 cc. of the liquid to be tested about 0.05 gram of peptone, 1 drop of 3 per cent ferric chloride solution, and 5 cc. of concentrated hydrochloric acid and heating to boiling. A violet color develops in the presence of formaldehyde.[3]

2. Gallic Acid Test.[4]—This test has been found by Sherman[5] to be a delicate and satisfactory test. Twenty-five to fifty cubic centimeters of the material should be acidulated with phosphoric acid and distilled. To the first 5 cc. of the distillate add 0.2 to 0.3 cc. of a saturated solution of gallic acid in pure ethyl alcohol and pour it cautiously down the side of an inclined test tube containing 3 to 5 cc. of pure concentrated sulphuric acid. If formaldehyde is present a green zone is formed at the junction of the two layers, gradually changing to a pure blue ring.

The delicacy of the test is about 1 part of formaldehyde in 500,000.

BENZOIC ACID AND SODIUM BENZOATE

Benzoic acid, in the form of its sodium salt, is probably the preservative most widely used in different varieties of food at present. This is partly due to its suitability for a wide range of products and partly to the fact that it is permitted in many food

[1] ROSENHEIM: *Analyst,* **1907,** 106.

[2] FULTON: *Ind. Eng. Chem., Anal. Ed.,* **1931,** 199.

[3] SALKOWSKI: *Z. Nahr.-Genussm.,* **1918,** 262.

[4] BARBIER and JANDRIER: *Ann. chim. anal.,* **1896,** 325; MULLIKEN and SCUDDER: *Am. Chem. J.,* **1900,** 444.

[5] *J. Am. Chem. Soc.,* **1905,** 1499.

products under the Federal pure food laws, provided that not more than 0.1 per cent is used and that the presence and amount are declared on the label.

Detection.—If the material is a liquid, 25 or 50 cc. may be acidified with 5 cc. of dilute sulphuric acid and shaken with 25 cc. of ether in a separatory funnel. In shaking, mix the liquids thoroughly by tipping the funnel back and forth ten or twelve times, but avoid *violent* shaking which tends to form emulsions, especially with saccharine liquids. If an emulsion forms, it can often be separated by drawing off the clear aqueous layer as much as possible and then giving the funnel a quick vigorous shake. If this does not suffice, add 10 cc. of gasoline or petroleum ether and shake again, or centrifuge the mixture in the machine described on page 34. The ether solution should be as clean and as free from the aqueous layer as possible.

If the original material is very thick or solid, a corresponding quantity should be diluted or macerated in a mortar with water and strained, to obtain a solution which is acidified and extracted as above.

The ether layer is separated and evaporated in a casserole or porcelain dish at room temperature or at a *low* heat. (*Do not bring it near a flame.*) If the original material contained much fat, which would be taken up by the ether, the preservative is best obtained by shaking the ether with dilute ammonia, removing the ammonia by evaporation, and testing the residue.

If considerable benzoic acid is present, it may be recognized in the residue from the ether as crystalline scales, having a characteristic odor when heated. The residue should be tested as follows:

Mohler's Method Modified by von der Heide and Jacob.[1]— Take up the ether residue in 1 to 3 cc. of a 0.33N sodium hydroxide and evaporate to dryness. To the residue add 5 to 10 drops of concentrated sulphuric acid and transfer as thoroughly as possible to a small test tube. Add a crystal of potassium nitrate and heat for 10 minutes in a glycerol or oil bath at 120 to 130°C., or for 20 minutes immersed in boiling water. In no case should the temperature exceed 130°C. The organic matter is oxidized and *m*-dinitro-benzoic acid is formed. After cooling, add 1 cc. of water and make decidedly ammoniacal; boil the solution to break

[1] *Z. Nahr.-Genussm.*, **1910**, 137.

up any ammonium nitrite that may have formed. Cool and add a few drops of fresh colorless ammonium sulphide, without allowing the layers to mix. A red-brown ring, due to the formation of ammonium *m*-diamidobenzoate, indicates benzoic acid. On mixing, the color diffuses through the whole liquid; on heating it finally changes to greenish yellow, owing to the decomposition of the amido salt.

Salicylic and cinnamic acids give a similar reaction except that the amido compounds formed do not decompose on heating, so that the change to the greenish-yellow color does not take place with these. Cinnamic acid, which may be present in the food as an oxidation product of the cinnamic aldehyde of cinnamon oil in the spice used, may be detected also by heating the ether residue to boiling with dilute chromic acid mixture. Then the cinnamic acid or cinnamic aldehyde will be oxidized to benzaldehyde, recognized by its characteristic almond odor.[1] It is to be remembered that the benzoic acid residue should be reasonably pure, since any large amount of organic matter interferes with the test. Phenolphthalein also interferes, but is not likely to be present in a food product.

Quantitative Determination.—The simplest method of determining small amounts of benzoic acid or benzoates in food is by extracting with a suitable immiscible solvent and titrating the extracted benzoic acid. Chloroform, while not so good a solvent for benzoic acid as ether, is preferable because it is not inflammable and dissolves only traces of mineral acids, tannin, salts, and other interfering substances. Further, it is heavier than water and can conveniently be drawn off from the bottom of the separatory funnel. By taking advantage of the "salting out" principle by using a saturated solution of sodium chloride, the benzoic acid is rendered much less soluble in the aqueous liquid and the extraction may be made quantitative.

Certain other organic acids, principally acetic, may be extracted in slight amount by the chloroform, but, if the extract is evaporated and dried, the error from this cause is negligible.

1. Preparation of the Solution.[2] *General Method.*—Grind in a sausage machine or food chopper if solid or semisolid and

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[1] Bigelow and Dunbar: U. S. Dept. Agr., *Bur. Chem. Bull.* **122**, p. 77.
[2] Dunbar: U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 138; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 433.

mix thoroughly. Transfer about 150 grams to a 500-cc. flask, add enough fine table salt to saturate the water in the sample, make alkaline to litmus paper with sodium hydroxide or milk of lime, and make up to the mark with saturated salt solution. Allow to stand at least 2 hours with frequent shaking and filter. If the sample contains large amounts of matter precipitable by salt solution, it is best to proceed as described under (*d*) of the Special Methods. If alcohol is present, follow the method described under (*c*) of the Special Methods. If much fat is present, it is well to make the filtrate alkaline and extract with ether before proceeding with the extraction of the benzoic acid.

Special Methods. *a. Ketchup.*—To 150 grams of sample, add 15 grams of fine table salt. Transfer the mixture to a 500-cc. graduated flask, using about 150 cc. of saturated salt solution for rinsing. Make slightly alkaline to litmus paper with 10 per cent sodium hydroxide and make up to 500 cc. with saturated salt solution. Allow to stand at least 2 hours with frequent shaking and filter through a large folded filter. If filtration is difficult, the mixture may be centrifuged before filtering.

b. Jellies, Jams, Preserves, and Marmalades.—Dissolve 150 grams of the sample in about 300 cc. of saturated salt solution. Add 15 grams of table salt. Make alkaline to litmus paper with milk of lime. Transfer to a 500-cc. graduated flask and fill to the mark with saturated salt solution. Allow to stand at least 2 hours with frequent shaking, centrifuge if necessary, and filter through a large folded filter.

c. Cider and Similar Products Containing Alcohol.—Make 250 cc. of the sample alkaline to litmus paper with sodium hydroxide and evaporate on the steam bath to about 100 cc. Transfer to a 250-cc. flask, add 30 grams of table salt, and shake until dissolved. Dilute to the mark with saturated salt solution, allow to stand at least 2 hours with frequent shaking, and filter through a folded filter.

d. Salted or Dried Fish.—Transfer 50 grams of the ground sample to a 500-cc. flask with water. Make slightly alkaline to litmus paper with 10 per cent sodium hydroxide and dilute to the mark with water. Allow to stand at least 2 hours with frequent shaking and filter through a folded filter. Measure 300

cc. of the filtrate into a second 500-cc. flask, add 90 grams of table salt, shake until dissolved, and fill to the mark with saturated salt solution. Mix thoroughly and filter off the precipitated proteins on a folded filter.

2. Extraction and Titration.—Measure a convenient portion (100 to 200 cc.) of the filtrate obtained by one of the above methods into a separatory funnel. Neutralize to litmus paper with hydrochloric acid (1:3) and add 5 cc. in excess. With salt fish, protein matter usually precipitates on acidifying, but this does not interfere with the extraction. Extract carefully with chloroform, using for a 200-cc. portion of the filtrate successive portions of 70, 50, 40, and 30 cc., and proportional amounts for smaller aliquots. To avoid emulsions, shake each time cautiously with a rotary motion; vigorous shaking is unnecessary. The chloroform layer usually separates readily (at the bottom) after standing a few minutes. If any emulsion forms, it can be broken by stirring the chloroform layer with a glass rod. If this does not succeed, draw off the emulsified portion into a second funnel and give it one or two sharp shakes. If this also fails, centrifuge the emulsion several moments. Since this is a progressive extraction, great care must be taken to draw off as much of the clear chloroform layer as possible each time, but on no account draw off any of the emulsion at any time. If not contaminated with the emulsion, it is not necessary to wash the chloroform extract.

Transfer the combined chloroform extracts to a shallow porcelain or glass dish, rinsing several times with small portions of chloroform, and evaporate to dryness at room temperature in a current of dry air. A blast of air that has passed through a bottle containing calcium chloride, or an electric fan, is convenient. If desired, a portion of the chloroform may be removed by distillation, taking care, by several rinsings with small amounts of chloroform, that no benzoic acid is lost in transferring back and forth. Dry the residue overnight (or until no odor of acetic acid can be detected, in the case of ketchup) in a sulphuric-acid desiccator. Dissolve the residue of benzoic acid in 30 to 50 cc. of neutral alcohol, add about one-fourth the volume of water, 2 drops of phenolphthalein solution, and titrate with $0.05N$ sodium hydroxide.

SALICYLIC ACID

Detection.—The solution of the food material for the qualitative test for salicylic acid may be prepared in the same manner as directed for the detection of benzoic acid, page 104, except that in no case should the amount of material used exceed 50 grams. Since the two preservatives occur in the same class of products, it is often advisable simply to divide the ether extract into two portions and test one for salicylic acid and the other for benzoic acid. In this case, since the test for benzoic acid is more difficult than that for salicylic, the larger portion should be tested for the former preservative. The portion of the ether extract that is to be tested for salicylic acid should be quite free from the aqueous layer, and, since the ferric test for salicylic acid is less delicate in the presence of mineral acids, it should be freed from traces of sulphuric acid by washing it twice with one-tenth its volume of water. Transfer the ether to a porcelain dish, evaporate it spontaneously or at low temperature, and test the residue by the following tests:

1. Ferric Chloride Test.—To the residue add 2 drops of dilute (1 per cent) ferric chloride solution, or better, 3 to 4 drops of ferric alum solution[1] and rub it around with a blunt glass rod.

A violet color is produced in the presence of salicylic acid. The test is delicate with ordinary care to 0.5 mg. of salicylic acid but is not conclusive of the presence of the preservative, since the color is not characteristic but is given by several other organic substances. In case of a positive result, therefore, the conclusion may be confirmed by the following test which is recommended by Sherman[2] as highly satisfactory:

2. Jorissen Test.[3]—Take up the portion of the ether residue that is to be used for this test in 10 cc. of hot water, transfer to a test tube, cool and add 4 or 5 drops of a 10 per cent solution of potassium (or sodium) nitrite, 4 or 5 drops of acetic acid, approximately 50 per cent strength, 1 drop of a 10 per cent solution of

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[1] Dissolve 2 grams of ferric alum in 100 cc. of water, heat to boiling and boil for a moment or two, allow to settle and filter. The reagent is preferable to ferric chloride in that a slight excess does not interfere with the delicacy of the test.

[2] "Organic Analysis," 2d ed., p. 381.

[3] JORISSEN: *Bull. l'acad. sci. Belg.*, **3**, 259; SHERMAN: *Ind. Eng. Chem.*, **1911**, 24; SHERMAN and GROSS: *ibid.*, **1912**, 492.

copper sulphate, mix and boil for $\frac{1}{2}$ minute; then let stand for 2 minutes. In the presence of salicylic acid the solution takes on a reddish tinge, and with more than a very minute amount becomes blood red. Phenol gives the same reaction but benzoic acid does not.

Notes.—A small amount of salicylic acid occurs naturally in some fruits; hence not more than 50 grams of food should be used if it is desired to show added salicylic acid.

As Sherman[1] carefully points out, it is not safe in testing foods to assume that a constituent volatile with steam, soluble in ether, capable of sublimation and crystallization, and giving a violet reaction with ferric salts, is necessarily salicylic acid. Highly roasted malt and baked food products which may have been partly caramelized have been reported to contain a substance ("maltol") which corresponds to salicylic acid in all these properties.[2] The Jorissen test, however, serves to distinguish salicylic acid from this and practically all the other substances that give a violet color with ferric chloride.

Quantitative Determination.—Salicylic acid is ordinarily added to foods in so much smaller quantity than benzoic acid that it is not feasible to determine it by titration. Recourse is therefore had to its colorimetric estimation, the method of extraction and estimation being very similar to the qualitative examination. The extracted salicylic acid must be carefully freed from accompanying substances, chiefly tannins, which give similar reactions and hence would interfere. For details of the procedure, which is a lengthy one and requires for good results some experience, reference is made to the directions of the Association of Official Agricultural Chemists.[3]

BORIC ACID AND BORAX

Qualitative Tests.—The well-known test with turmeric paper is the most convenient as well as the most delicate for the detection of boric acid and borates in food products. It may be carried out in the following manner:

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[1] *Loc. cit.*

[2] BRAND: *Z. ges. Brauw.*, **15**, 303; BACKE: *Ann. des fals.*, November, **1909**; SHERMAN: *Ind. Eng. Chem.*, **1911**, 24.

[3] "Official Methods of Analysis," **1935**, p. 431.

Make about 25 grams of the sample distinctly alkaline with lime water or sodium hydroxide, and evaporate to dryness in a porcelain or platinum dish. Ignite the residue until charred, boil with about 15 cc. of water, add hydrochloric acid, drop by drop, until acid to litmus paper, then add 10 drops in excess. Filter and evaporate to dryness on the water bath with a strip of turmeric paper immersed partly in the liquid. In the presence of borax or boric acid, the dry paper will be colored cherry red, which is changed by a drop of ammonia to bluish green.

Note.—By carrying out the evaporation and drying at room temperature in a desiccator, the test can be made considerably more delicate,[1] but this increased delicacy is of little value if it is desired to test only for boric acid *added as a preservative.* Boric acid in small quantities is widely distributed in nature, apples and other fruits and vegetables showing distinct traces of it, up to 0.025 per cent having been reported. It occurs also in common salt, some kinds of which contain considerable amounts. In doubtful cases, therefore, it is best to determine the boric acid quantitatively.

Quantitative Determination.[2]—To 50 or 100 grams of the sample add enough sodium hydroxide (1 or 2 grams) to render it distinctly alkaline and evaporate to dryness in a platinum dish. Cautiously, but thoroughly, char the residue (it is not necessary to burn all the carbon) and boil with 20 cc. of water, adding hydrochloric acid drop by drop until distinctly acid. Filter into a 100-cc. volumetric flask and wash with a little hot water, keeping the volume of filtrate to 50 to 60 cc. Return the filter to the platinum dish, moisten it thoroughly with lime water, dry on the steam bath and ignite to a white ash. Dissolve this in a few cubic centimeters of dilute hydrochloric acid and rinse with a little water into the 100-cc. flask. Add 0.5 gram of dry calcium chloride, a few drops of phenolphthalein indicator, then a 10 per cent solution of sodium hydroxide cautiously until a slight permanent pink color is produced, and finally make up to 100 cc. with lime water. All the phosphoric acid has thus been precipitated as calcium phosphate. Shake and filter through a dry filter. To 50 cc. of the filtrate add normal sulphuric acid until the pink color just disappears; then add a few drops of methyl red

[1] Low: *J. Am. Chem. Soc.*, **1906**, 805.
[2] Thomson: *Glasgow City Anal. Soc. Rept.*, **1895**, 3.

and continue the addition of sulphuric acid until the solution is just turned to pink. At this point all the acids are present as salts neutral to phenolphthalein except boric acid and carbonic acid. Boil the solution 2 minutes to expel carbon dioxide. Cool the solution and add $0.1N$ sodium hydroxide until it is just not acid to methyl red. Add a little more phenolphthalein, 1 to 2 grams of neutral mannitol, and titrate with $0.1N$ sodium hydroxide until pink. Add a little more mannitol and, if the pink color disappears, add the standard sodium hydroxide until again pink. Repeat the addition of mannitol and sodium hydroxide if necessary until a permanent pink color is produced. Each cubic centimeter of sodium hydroxide used in the final titration is equal to 0.0062 gram of boric acid.

Notes.—Although alkali is added before ignition, there is a possibility of some boric acid being volatilized through imperfect mixing of the alkali with the food. Hence it is best to ignite no more than is necessary to char the material thoroughly in order that it may be exhausted with water.

The method described is practically the original method of Thomson, as described in the "Official Methods" of the Association of Official Agricultural Chemists (1935), except that methyl red is substituted for methyl orange and mannitol for glycerol. It has been repeatedly shown, however, to be subject to considerable error when applied to food products containing large proportions of phosphates and fat with small amounts of boric acid, such as might occur in some natural products. These errors are commonly precipitation of some calcium borate along with the phosphate, incomplete removal of phosphate, or volatilization of boric acid with glycerol coming from the fat during the alkaline ashing. If the conditions mentioned above are known to exist, the method may be modified as suggested by Monier-Williams[1] by avoiding the use of lime and precipitating the phosphates with magnesia mixture, or, as proposed by Alcock,[2] the boric acid is converted into its methyl ester, distilled in a special apparatus, and the boric acid finally titrated as in the Thomson method.

Boric acid is a very weak acid, requiring for its direct titration an indicator changing at pH 11, but forms with polyhydric

[1] *Analyst,* **1923,** 413.
[2] *Analyst,* **1937,** 522.

alcohols, as mannitol,[1] $C_6H_8(OH)_6$, complexes that have much larger ionization constants than boric acid itself, enabling the use of phenolphthalein indicator at pH 8–9. A volume of glycerol, neutral to phenolphthalein, equal to the volume of the solution to be titrated, as used originally by Thomson, may be substituted for the mannitol if desired, or 10 to 15 cc. of invert sugar solution[2] (due to its content of fructose).

Electrometric titration curves[3] have shown that of these polyhydric alcohols mannitol gives the sharpest change. In each case the boric acid complex acts as a monobasic acid; hence its equivalent weight is its molecular weight.

Sulphurous Acid and Sulphites

Sulphurous acid is generally employed in the preservation of food products in the form of acid sulphites, especially of sodium or calcium, or it may be present in the form of organic sulphur dioxide combinations. This is the case especially with such products as wines or molasses where the acid is probably present partly in combination with aldehydes or sugars. There is no simple reliable qualitative test, and a determination, at least roughly quantitative, is generally made.

Detection and Determination.—Two quantitative methods are described here, the first of which is simpler and more rapid and will be found satisfactory for many food products.

Direct Distillation.—The apparatus used is practically the same as that employed in a Kjeldahl ammonia distillation, or it can be readily devised from laboratory apparatus. Figure 40 (p. 113) shows a simple setup of this kind.[4] It consists essentially of an 800-cc. Kjeldahl flask attached to an efficient condenser. The safety tube is somewhat flared at the top and drawn out to a point at the lower end. To the lower end of the

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[1] Vedam: *J. pharm. chim.*, VI, **8**, 109; Jones: *Am. J. Sci.*, **1899**, 147.

[2] Gilmour: *Analyst*, **1921**, 3; **1924**, 576. The invert sugar reagent is prepared as follows: 25 grams of cane sugar is heated with 10 cc. of water in a small round-bottomed flask until completely dissolved; the solution is boiled for a few minutes. Add 1 cc. of 0.5N hydrochloric acid to the hot solution and shake well without reheating. Dilute, cool, add 1 cc. of 0.5N sodium hydroxide, and make up to 50 cc. The resulting reagent should be practically neutral and colorless.

[3] Mellon and Morris: *Ind. Eng. Chem.*, **1924**, 123.

[4] Nichols and Reed: *Ind. Eng. Chem., Anal. Ed.*, **1932**, 79.

condenser is closely attached a small straight calcium chloride tube, closely fitting the Erlenmeyer flask and dipping into 50 cc. of the oxidizing solution, which may be either saturated bromine water or standardized iodine solution. In the latter case the flask should stand in ice water.

Method.—Weigh 25 to 100 grams of the sample into the Kjeldahl flask, add 300 cc. of distilled water, and connect with the condenser. Add through the safety tube 10 cc. of 10 per cent sodium acid carbonate solution, followed in 1 to 5 minutes by 10 cc. of concentrated hydrochloric acid.

Distill about 150 cc. in approximately 45 minutes. After the distillation is complete the excess of iodine may be titrated if desired, but a gravimetric determination of the sulphuric acid is preferable. Boil off the excess of iodine or bromine, dilute the solution to 250 cc., add 5 cc. of hydrochloric acid (sp. gr. = 1.12), heat to boiling, and add very slowly, with constant stirring, a hot 10 per cent solution of barium chloride until in slight excess. Allow to stand for 1 hour, filter, wash with hot water, ignite, and weigh as barium sulphate.

A "blank" determination should be run with none of the food material in the flask, and any barium sulphate obtained subtracted from the main amount. If no precipitate is obtained or if no more than is found in the
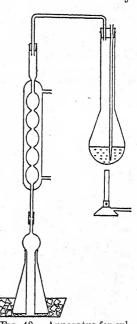
Fig. 40.—Apparatus for sulphur dioxide distillation.

blank, added sulphur dioxide is absent. The amount found normally is in the neighborhood of 5 to 50 mg. as SO_2. Hydrochloric acid has been substituted for the phosphoric acid formerly used because, when the sulphur dioxide is in close combination with the sugars, it effects a better liberation of the gas.

Notes.—This method determines the *total* sulphurous acid, both free and combined. If it is desired to determine the free acid only, the same procedure may be used, except that no acid is added.

With food products containing large quantities of volatile sulphur compounds, such as onions, mustard, relishes, etc., the method described above is quite inaccurate on account of the distillation and oxidation of these substances. Better results are secured by the method of Monier-Williams,[1] which, as modified by Fitelson,[1] has been adopted by the Association of Official Agricultural Chemists. Although time-consuming it has the advantage of more thorough liberation of the sulphur dioxide, avoiding errors due to volatile sulphur compounds and permitting a volumetric determination.

Monier-Williams Method.—*Principle.*—The sample is distilled for a given length of time under a reflux condenser, and the sulphur dioxide is swept into cold hydrogen peroxide by a current of carbon dioxide. Hydrochloric acid is used to acidify the product, the reflux condenser preventing the acid from distilling over. The cold hydrogen peroxide oxidizes the sulphurous acid to sulphuric acid quantitatively, thus permitting the final determination of the sulphur dioxide in the product either by the gravimetric barium sulphate precipitation or by the titration of the sulphuric acid with a standard solution of sodium hydroxide.

Procedure.—Connect a 750-cc. round-bottomed Pyrex flask, *B*, Fig. 41, to a sloping reflux condenser *D*, the lower end of which is cut off at an angle. Or an upright two-necked round-bottomed flask can be used. Pass carbon dioxide from a generator through a sodium carbonate solution in *A* to remove chlorine, also connect a dropping funnel *K* to *B* by the three-holed stopper *C*. Connect the upper end of the condenser by the tube *E* to a 200-cc. Erlenmeyer flask *F*, which is followed by a Peligot tube *G*. This delivery tube *E* extends to the bottom of the receiver. One Peligot tube is sufficient to catch traces of sulphur dioxide swept through the flask *F*. Rubber stoppers are used throughout. The receiver *F* contains 15 cc. of pure, neutral 3 per cent hydrogen peroxide, while the Peligot tube contains 5 cc. Hydrogen peroxide usually contains free sulphuric acid. Start with 30 per cent hydrogen peroxide, dilute somewhat, and neutralize with barium hydroxide solution, using bromophenol blue as indicator. After the reagent has settled in the cold, filter from the barium sulphate, determine its strength by permanganate

[1] Monier-Williams: *Public Health Rept.* 43, Ministry of Health, London, 1927; Fitelson: *J. Assoc. Off. Agr. Chem.*, **1929**, 120.

titration, and finally adjust to 3 per cent. The bromophenol blue indicator in the hydrogen peroxide remains unaffected for some time.

After connecting the apparatus, place 300 cc. of distilled water and 20 cc. of concentrated hydrochloric acid in the flask and boil for a short time in a current of carbon dioxide. Now introduce the food to be tested, the procedure depending on the food. Add liquids directly by means of the dropping funnel. In the
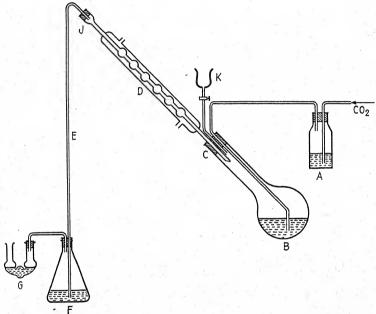
Fig. 41.—Monier-Williams apparatus for sulphur dioxide.

case of easily transferable solids, first cool the flask contents somewhat, taking care by regulating the flow of carbon dioxide to avoid having the hydrogen peroxide drawn up in the delivery tube *E*. Then quickly introduce the food by removing the stopper *C*. With semisolid foods, requiring more time to introduce into the flask, cool the contents of the flask by gradual immersion in cold water and wash the food in quickly with recently boiled distilled water. After introducing the food, boil the mixture for 1 hour (1½ hours in the case of dried fruits) in a slow current of carbon dioxide, stopping the flow of water in

the condenser just before the end of the distillation. This causes the condenser to become hot and drives over residual traces of sulphur dioxide retained in the condenser. When the delivery tube just above the receiver E becomes hot to the touch immediately remove the stopper J.

Wash the delivery tube and the Peligot tube contents into the flask F and titrate the liquid at room temperature with $0.1N$ sodium hydroxide, bromophenol blue being used as indicator. The sodium hydroxide must be standardized with this indicator. Bromophenol blue is unaffected by carbon dioxide and also gives a distinct color change in cold hydrogen peroxide. One cubic centimeter of $0.1N$ sodium hydroxide is equivalent to 3.2 mg. of sulphur dioxide, so that titration of small quantities of sulphur dioxide, requiring less than 0.5 cc. of sodium hydroxide, is not accurate. A gravimetric determination may be made after titration, the precipitation of barium sulphate being made at room temperature. After settling, filter the supernatant liquid and wash the residual barium sulphate three times by decantation with boiling water. Determine a blank on the reagents, both by titration and gravimetrically, and correct the results accordingly.

Formic Acid

Formic acid has found some application in recent years as a preservative for fruit products, and its efficiency is rather surprisingly high, comparing favorably with salicylic acid.[1] Small amounts of formic acid may also occur naturally in some foods, since it is formed by the fermentative action of bacteria and yeasts on carbohydrates. The amounts commonly found when no formic acid has been added run less than 10 mg. per 100 grams of material. Substances containing considerable caramelized carbohydrate, as roasted coffee or cereals, may show over 100 mg. Manufactured sugar products, as molasses and refinery sirups, may run as high as 800 mg., owing largely to the action of alkali on the reducing sugar present.[2]

Detection.—Mix 50 grams of the material with 20 cc. of 20 per cent phosphoric acid and distill by steam until about 200 cc. of distillate is obtained, gently heating the flask containing

[1] Smith: *J. Am. Chem. Soc.*, **1907**, 1236.
[2] Zerban: *J. Assoc. Off. Agr. Chem.*, **1932**, 355.

the acid mixture in order to avoid condensation and keep the volume approximately constant. Test the distillate by the following tests:

1. Test 5 to 10 cc. of the mixed distillate for formaldehyde by either of the two tests on pages 102 and 103. If formaldehyde is absent, subject 25 to 50 cc. of the distillate to the test below.

2. *Reduction to Formaldehyde.*[1]—Add to a portion of the distillate dilute (1:4) sulphuric acid and enough magnesium filings to produce a vigorous but not violent evolution of hydrogen. Allow the action to continue for 1 hour and test the solution for formaldehyde as suggested under (1).

Determination.—Use 25 or 50 grams of the sample, dilute to 100 cc. and carry out the determination as described on page 432. In using this method, which is described as applied to vinegar, for food products in general, the following additional precautions should be noted.[2] The volume of the sample and of the carbonate mix should be kept approximately constant to avoid caramelization. Not less than 1,000 cc. of distillate is to be collected, and the sample must contain no free mineral acid. If the material is likely to contain sulphur dioxide, 2 grams of barium carbonate, the sulphite of barium being less soluble, should be substituted for the calcium carbonate.

FLUORIDES

The fluorides of sodium and ammonium have been used to some extent as food preservatives, especially at one time in beer and malt extracts. Fluorides and fluosilicates are also used quite extensively as insecticides and as dusting powders on growing crops. The presence of even minute traces of fluorides in drinking water and possibly in some foods produces "fluorosis," which shows as "mottled teeth" in growing children. The most common method for their detection is by the well-known etching of glass.

Method.—If the sample is a liquid, use 150 cc. of it. Add 10 cc. of potassium sulphate (33 grams per liter), heat the solu-

[1] FENTON: *J. Chem. Soc.*, **1907**, 687; BACON: U. S. Dept. Agr., *Bur. Chem. Circ.* **74**, p. 6.

[2] *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 442; SEEKER: *J. Assoc. Off. Agr. Chem.*, **1915**, 210.

tion to boiling, and while boiling add drop by drop 10 cc. of a 10 per cent solution of barium acetate. Boil for 1 minute, allow to settle, and decant off the clear supernatant liquid. Wash the precipitate once with water by the aid of a centrifuge, thus avoiding the use of filter paper, which sometimes contains traces of fluorides, transfer to a platinum or nickel crucible, dry, and ignite gently to destroy organic matter.

If the substance is a solid, mix it with a small quantity of sodium carbonate and burn to an ash; then proceed with the addition of sulphuric acid, as directed below.

Meanwhile a small glass plate, clear and free from scratches, is thoroughly cleaned and coated on one side with a mixture of equal parts of paraffin and carnauba wax. This can readily be done by pouring a little of the melted wax on the warmed plate. If the excess is drained off and the glass held level, a thin uniform wax coating will result. While the coating is still warm, make with a pointed instrument a characteristic mark, such as a small cross ($+$), in the wax, taking care that the glass is laid bare but not scratched. The precaution should be taken to have the cross of approximately the same size in the different tests; for example, with arms about 4 mm. long and 1 mm. wide. On the uncoated side of the plate locate the cross by marks placed at the ends of the arms with a diamond or file.

To the precipitate in the crucible add 2 or 3 cc. of concentrated sulphuric acid, hold the crucible in the tongs, and heat its upper edge cautiously and quickly in a small flame. Press the glass plate quickly down on the warm crucible, having the cross nearly in the center, and hold it a moment to seal the glass securely to the crucible. The crucible should be embedded in the wax so firmly that it can be lifted by the plate. Support the crucible in a piece of heavy asbestos board in which a hole has been cut so that the crucible fits closely; put 2 or 3 drops of water on the glass plate and press down on it a condenser, the lower end of which is closed by a piece of thin sheet rubber such as is employed by dentists. Heat the crucible for 1 hour by a small flame about 9 mm. long, placed 6 mm. below the bottom of the crucible. At the end of this time remove the plate, scrape off the wax, and clean the glass on both sides with "bon ami" or other polishing material that will not scratch the glass. Examine it

by reflected light for an etching. A test should not be considered positive unless the cross can be seen when viewed from either side of the glass.

Notes.—It is hardly necessary to speak of the need for using the purest reagents obtainable and testing them carefully by blank tests.

The condenser may be made from a piece of wide glass tubing or a "carbon funnel" arranged so that a constant current of cold water may be kept flowing through it and with a piece of thin sheet rubber (dental dam) stretched tightly over the bottom. The diameter of the tube should be somewhat greater than that of the platinum crucible.

The method described is applicable to soluble fluorides only and is not satisfactory if a great deal of silica is present. In this case the hydrofluoric acid would form silicon fluoride and thus escape detection. In such case reference may be made to the "Official Methods" of the Association of Official Agricultural Chemists (**1935**, p. 439) or to Jacobs, "Chemical Analysis of Foods and Food Products," for the necessary modifications of the test to include insoluble fluorides (fluoborates, fluosilicates, etc.).

The delicacy of the procedure above described, as tested on aqueous solutions of potassium fluoride, is rather surprising. A perfectly distinct etching is readily obtained from 150 cc. of solution containing 1:10,000,000 of fluorine, and by careful working it is possible to get a recognizable test from 1:50,000,000. By a recognizable test is meant one that is visible from either side of the glass and does not have to be brought out by breathing on the glass. Care should be taken to clean the glass with "bon ami" before examining it, since sulphuric acid alone will give a "stain" which can be brought out by breathing on it, and might easily be mistaken for an etching. The scouring will always remove this, however.

The amounts of fluorides found in ordinary foods are usually of the order of 0 to 2, expressed as parts per million of fluorine. Tea is exceptionally high, often being in the neighborhood of 100 parts or more. Vegetables sprayed with insecticides have been found to contain up to 75 parts; some of the chemical substances used in foods, as in baking powders, run from 6 to 16; 1.4 parts per million is the present tolerance of the U. S. Food and Drug Administration.

It is possible, by controlling more closely the temperature during the etching, to make the method approximately quantitative, and this should be done in doubtful cases on account of the wide distribution of traces of fluorine in natural products.[1]

If a strict quantitative determination is necessary, the method most commonly employed is that of Willard and Winter[2] based on the separation of fluorine by distillation as hydrofluosilicic acid, in which the fluorine may be determined colorimetrically by its bleaching action on a mixture of zirconium nitrate and alizarin sulphonic acid, or by titration with thorium or cerous nitrate. Details of the method, which is best suited for more than 5 parts per million of fluorine, may be found in the references cited. A very good critical discussion of the most useful methods is given by Lockwood.[3]

SACCHARIN

Saccharin, or benzoyl sulphimide, $\begin{matrix} & CO \\ & \diagdown \\ & NH, \\ & \diagup \\ & SO_2 \end{matrix}$

although better known as the first of the artificial sweeteners to be used commercially, possesses a certain degree of antiseptic power and hence may be included among the preservatives. The Referee Board of Consulting Scientific Experts having found that the continued use of saccharin for a long time, in quantities over 0.3 gram per day, is liable to impair digestion, its use in foods has been prohibited under the Federal food laws.[4]

The simplest method of testing for saccharin is to extract the material with ether and note whether the residue left after evaporating the ether has an extremely sweet taste. A more delicate test is to convert the saccharin to sodium salicylate and test for salicylic acid. This latter test obviously can be applied only in the absence of salicylic acid. There is occa-

[1] WOODMAN and TALBOT: *J. Am. Chem. Soc.*, **1906**, 1438; **1907**, 1362.

[2] *Ind. Eng. Chem., Anal. Ed.*, **1933**, 7; WINTER: *J. Assoc. Off. Agr. Chem.*, **1936**, 362.

[3] *Analyst*, **1937**, 775.

[4] U. S. Dept. Agr., *Food Inspection Decision* **135**.

sionally present in some food materials a substance, the so-called "false saccharin," which also responds to this test and must be removed.

Preliminary Test.—Extract with ether as described under salicylic acid. Evaporate the ether spontaneously and taste of the residue. The residue will taste sweet in the presence of saccharin to the amount of 20 mg. per liter.

Confirmatory Test.—Acidify 50 cc. of a liquid food or the water solution of 50 grams of a solid, prepared as directed under salicylic acid, and extract with ether. Test the ether extract for salicylic acid as described on page 108. If petroleum ether was used to purify the residue, return the petroleum ether to the dish containing the residue, evaporate, dilute to about 10 cc., and add 2 cc. of sulphuric acid (1:3). Bring the solution to the boiling point and add a 5 per cent solution of potassium permanganate, drop by drop, to slight excess. Partly cool the solution, dissolve in it a piece of sodium hydroxide and filter into a small silver dish (a porcelain crucible can be used). Evaporate to dryness and heat for 20 minutes at 210 to 215°C., using an oil or air bath. Dissolve the residue in water, acidify, and extract with ether, evaporate the ether, and test the residue with 2 drops of a 2 per cent solution of ferric alum.

Note.—By this method all the so-called "false saccharin" and the salicylic acid naturally present (also added salicylic acid when not present in too large amount) are destroyed, while 5 mg. of saccharin per liter can be detected with certainty.

For the quantitative determination of saccharin, it may be fused with sodium carbonate and calculated from the sulphur content, weighed as barium sulphate,[1] or it may be hydrolyzed to ammonia by evaporation in the presence of hydrochloric acid. The resulting concentration of ammonium chloride is determined by nesslerization and comparison with standard ammonium chloride solutions.[2]

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[1] *Assoc. Off. Agr. Chem.*, "Official Methods," **1935,** p. 435.
[2] Guiseppi: *Z. Nahr.-Genussm.* **1909,** 577; Krantz: *J. Assoc. Off. Agr. Chem.*, **1934,** 194.

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CHAPTER IV

MILK AND CREAM; ICE CREAM

Milk

Milk is a food material of complex and variable composition but can be described as essentially an aqueous solution of milk sugar, mineral salts, and soluble albumin containing suspended globules of fat and partially dissolved casein.

Of these constituents, *milk sugar* or lactose is more fully discussed in the chapter on Carbohydrate Foods, page 255, and the *fat* is treated separately under Butter, page 225. The *casein*, which comprises approximately 80 per cent of the total protein of milk, is believed to be present in combination with calcium, and is not in solution but as a colloidal dispersion, *i.e.*, in the form of very small gelatinous suspended particles. By the action of dilute acids or of rennet, due primarily to the enzyme *rennin*, the free casein is precipitated. Free casein is readily soluble in dilute alkalies, from which it is precipitated by adding acid. Its alkaline solutions are levorotatory, having a specific rotation of about $-90°$. The soluble albumin (lactalbumin) makes up by far the greater part of the remaining protein and is quite similar to the albumin of egg, being readily soluble in water, coagulated by heating to 75°C., and having a specific rotation of about $-68°$. The mineral salts existing in milk are to a certain extent conjectural, since the salts found by analysis of the ash are not exactly the same as those present in the milk itself. They consist largely, however, of the chlorides, phosphates, and citrates of sodium, potassium, calcium, and magnesium. Of the other substances that occur in milk, mostly in very small amounts, citric acid, which is present up to 0.15 per cent in the form of citrates, is the only one that needs to be mentioned.

In short, milk is probably the most studied, as well as the most variable, food product, about which a veritable mass of information has been gathered, of which only typical summaries can be given here.

Composition

General Composition.—In approximate figures the average percentage composition of milk may be stated:

	Per cent
Total solids	12.8
Fat	4.0
Protein	3.3
Ash	0.7
Milk sugar	4.8
Solids not fat	8.8

From these figures there may be in normal milk quite decided variations, and figures have been reported that differ widely from them, some of the discrepancies of the older analyses being undoubtedly due to the imperfect methods of analysis employed.

Lythgoe[1] states that all milk completely drawn from healthy cows will fall between the following limits:

TABLE 9

Composition	Extreme limits, per cent	Usual limits, per cent	Herd milk, per cent
Total solids	10.0–17.0	10.5–16.0	11.8–15.0
Fat	2.2– 9.0	2.8– 7.0	3.2– 6.0
Protein	2.1– 8.5	2.5– 4.5	2.5– 4.0
Ash	0.6– 0.9	0.7– 0.8	0.7– 0.8
Milk sugar	4.0– 6.0	4.2– 5.5	4.3– 5.3
Solids not fat	7.5–11.0	7.7–10.0	8.0– 9.5

Variations in Composition.—Besides variations in composition which may be due to individual cows, there are also certain well-established differences due to environment or to racial influences. Among the more important of these are:

a. The Breed of the Cow.—Some breeds yield quantity, others quality. The Jersey and Guernsey cattle, for instance, give comparatively small quantities of milk rich in fat; the Holstein cows, on the other hand, yield much larger amounts of milk of decidedly lower solids and fat content. These differences are well summarized in the following table based on data collected by the Massachusetts Board of Health.[2]

[1] *Mass. State Bd. Health Bull.*, **1910**, p. 419.
[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 129.

If individual differences are eliminated and only fully drawn mixed milk from herds is considered, the variation due to breed

TABLE 10

Breed	Specific gravity	Total solids, per cent.	Fat, per cent.	Protein, per cent.	Ash, per cent.	Solids not fat, per cent.	Milk sugar, per cent.
Jersey..........	1.034	14.57	5.40	3.54	0.78	9.17	4.85
Guernsey.......	1.034	14.40	5.00	3.77	0.77	9.40	4.86
Ayrshire........	1.032	12.57	4.00	2.90	0.77	8.57	4.90
Dutch Belt.....	1.032	12.03	3.60	2.62	0.68	8.43	5.00
Holstein........	1.032	11.96	3.35	2.99	0.69	8.61	4.89

is the factor of the greatest influence in permanently affecting the composition of milk.

b. The Time of Year.—The poorest milk is produced during the spring and early summer months, the richest during the seasons of autumn and early winter, when the cattle are getting a smaller proportion of green feed. This difference is clearly shown in the following table[1] which gives the seasonal average for 16 years:

Time of year	Total solids, per cent	Fat, per cent	Solids not fat, per cent
November–January...............	13.04	4.11	8.93
February–April..................	12.72	3.88	8.84
May–August.....................	12.66	3.89	8.77
October–November..............	13.03	4.25	8.78

This variation in composition of milk between the pasture-fed and the stall-fed season has in the past received legal recognition in the fixing of milk standards. In Massachusetts, for many years, the legal standard for total solids was set at 13 per cent in the winter months and at 12 per cent in the summer season.

c. Time of Day.—Milk that has been drawn in the evening is nearly always richer in fat than the morning milk, as shown in the following averages:

[1] RICHMOND: "Dairy Chemistry," p. 126.

Time of day	Specific gravity	Total solids	Fat
Morning milk.....................	1.0322	12.53	3.63
Evening milk....................	1.0318	12.94	4.04

d. "Fore" Milk vs. "Strippings."—If different portions of the whole quantity of milk obtained at a single milking are examined separately they will be found to show marked differences in fat content, especially as between the first and last portions. The other constituents of the milk do not vary so greatly as the fat. The first portions of milk, the "fore" milk, contain much less fat than do the last portions or "strippings." The following figures, due to Van Slyke, illustrate this point:

Portion	Fat in milk, per cent		
	Cow 1	Cow 2	Cow 3
First portion drawn...............	0.90	1.60	1.60
Second portion drawn............	2.60	3.20	3.25
Third portion drawn.............	5.35	4.10	5.00
Fourth portion drawn (strippings)..	9.80	8.10	8.30

This difference in composition is explained by the separation of the milk while in the udder of the cow, cream rising to the top just as would happen if the milk stood in a vessel, hence being drawn last. Dishonest dairymen have in the past taken advantage of this fact in adulteration cases by having the cows *partially* milked in the presence of unsuspecting witnesses, the resulting "known purity" milk being thus largely "fore" milk.

In general it will be found that to whatever causes the variations noted in the composition of milk are due, the differences are shown much more in the fat than in any other constituent. The protein is also variable, although to a somewhat less extent, and the milk sugar and ash are much more nearly constant.

METHODS OF ANALYSIS

Preparation of the Sample.—Since the cream will rise on a sample of milk sufficiently in 5 minutes to destroy the uniformity

of the sample, great care must be used in taking a portion for analysis to ensure that it represents a fair average of the milk. The best way is to pour the milk from the containing vessel into another and back again several times, or if this is impracticable it should be thoroughly stirred before being sampled. If the analytical sample has stood for any appreciable time it should be mixed by pouring back and forth before a portion is removed to test; otherwise concordant results cannot be obtained. Do not shake the sample, since this tends toward a separation of the fat.

Specific Gravity.—This is usually taken with a special form of hydrometer known as a *lactometer* (Fig. 42). The Quevenne lactometer has a scale graduated into 25 equal parts, extending from 15 to 40, corresponding to specific gravities from 1.015 to 1.040. The best form of instrument is that provided with a thermometer.

The lactometer is graduated to give correct results at 60°F. (15.6°C.) and the reading should be made at approximately that temperature, between 55 and 65°, and then corrected to standard temperature. This may be done by adding 0.1 to the reading for each degree Fahrenheit above 60°F., or subtracting 0.1 for each degree Fahrenheit below 60°F. If the temperature is read in Centigrade degrees the correction may be made by Table 11, page 128.

The New York Board of Health lactometer has a scale reading 0 in water, and 100 in milk with a specific gravity of 1.029, which is taken as the lowest limit for pure milk. The instrument is used in the same manner as the Quevenne lactometer and the readings can be converted into degrees of the latter instrument by multiplying by 0.29.

Fig. 42.—Lactometers.

Notes.—The specific gravity of milk fat is about 0.93; of the solids not fat approximately 1.5. The specific gravity of the milk itself is thus a function of the two; the former lowers it, the latter increases it. As would be expected from the variable composition of milk, the specific gravity is also a variable. The

values for normal milk from a herd, however, will usually fall between 1.030 and 1.034.

TABLE 11.—FOR CORRECTING THE SPECIFIC GRAVITY OF MILK ACCORDING TO TEMPERATURE. ADAPTED FROM THE TABLE OF VIETH

(Temperature in Degrees Centigrade)

Specific gravity	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°	20°
1.025	24.1	24.3	24.5	24.6	24.7	24.9	25.1	25.3	25.4	25.6	25.9
26	25.1	25.2	25.4	25.5	25.7	25.9	26.1	26.3	26.5	26.7	27.0
27	26.1	26.2	26.4	26.5	26.7	26.9	27.1	27.4	27.5	27.7	28.0
28	27.0	27.2	27.4	27.5	27.7	27.9	28.1	28.4	28.5	28.7	29.0
29	28.0	28.2	28.4	28.5	28.7	28.9	29.1	29.4	29.5	29.8	30.1
30	29.0	29.1	29.3	29.5	29.7	29.9	30.1	30.4	30.5	30.8	31.1
31	29.9	30.1	30.3	30.4	30.6	30.9	31.2	31.4	31.5	31.8	32.2
32	30.9	31.1	31.3	31.4	31.6	31.9	32.2	32.4	32.6	32.9	33.2
33	31.8	32.0	32.3	32.4	32.6	32.9	33.2	33.4	33.6	33.9	34.2
34	32.7	33.0	33.2	33.4	33.6	33.9	34.2	34.4	34.6	34.9	35.2
35	33.6	33.9	34.1	34.4	34.6	34.9	35.2	35.4	35.6	35.9	36.2

Directions.—Find the observed gravity in the left-hand column. Then, in the same line, and under the observed temperature will be found the corrected reading.

Taken by itself the specific gravity is of little value in showing adulteration. The addition of water lowers the specific gravity of milk; the removal of cream raises it, this being the lighter portion of the milk. It is therefore theoretically possible by skillful manipulation to both skim and water a sample and still have its specific gravity correspond to that of normal milk. Such a sample would, however, be readily recognized by one familiar with the appearance of the genuine product.

The lactometer reading is of value in the rapid analysis of milk for calculating the solids in connection with the Babcock method of fat determination (see page 136).

Acidity.—Measure 25 cc. of milk into a 125-cc. flask, and add an equal volume of water that has been recently boiled and cooled, to free it from carbon dioxide. Titrate with $0.1N$ sodium hydroxide, using 0.5 cc. of phenolphthalein indicator. Each cubic centimeter of the base equals 0.0090 gram of lactic acid.

Total Solids.—Use a platinum dish having a flat bottom about $2\frac{1}{2}$ in. in diameter. Ignite and weigh the dish accurately, then add about 5.1 grams to the weights on the balance pan. With a pipette deliver 5 cc. of the well-mixed milk into the dish and weigh the whole as rapidly as possible to the nearest milligram.

Evaporate the milk for ½ hour on the water bath and then dry it in the oven at 100°C. for 3 hours.

Notes.—It is important that the milk should be dried in a thin layer, so that the removal of the water will take place as quickly as possible. Under these conditions the residue obtained is nearly white, but if the process be prolonged, it may have a brownish color from the caramelization of the sugar. The drying may be helped by spreading in the dish, prior to weighing, 15 to 20 grams of pure dry sand.

Aluminum or lead foil dishes or tin blacking-box covers may be used instead of platinum dishes. In this case, however, or if sand is used, the ash determination must be done on a separate sample.

For the determination of the total solids by calculation from the fat and lactometer reading see page 136.

Ash.—Ignite the platinum dish containing the residue from the preceding determination at a low red heat until the ash is white or of a uniform light-gray color. This may be done in a muffle furnace at a temperature not exceeding 600°C., or over a burner carefully regulated so that the dish is nowhere heated above the slightest visible redness. If the method used for total solids does not permit of direct ignition, weigh quickly about 20 grams of milk into a weighed platinum or porcelain dish, add 6 cc. of strong nitric acid, evaporate to dryness on the steam bath, and proceed as before.

The ash, after weighing, may be tested for boric acid as described on page 110.

Fat.—The time-honored method for determining the fat is the Adams process, in which the milk is dried on porous paper and the fat extracted with ethyl ether or petroleum ether in a continuous extraction apparatus. Since the method is tedious and is not well suited for milk products other than liquid milk, it is not described in detail.

1. *Babcock Method.*—Measure 17.6 cc. of milk from a pipette into the graduated test bottle; add 17.5 cc. of sulphuric acid (sp. gr. 1.825), pouring it in slowly so as to form a layer beneath the milk. After the acid has thus been added to all the bottles mix the milk and acid thoroughly by a rotary motion until all traces of curd have disappeared, avoiding the spurting of the liquid into the neck of the bottle. Place the bottles in opposite

pockets of the centrifuge, in even numbers, and whirl them for 5 minutes at the proper speed.   The correct speed varies from 1,000 revolutions per minute for a 10-in. wheel to 700 for one of 24 in. diameter.   Then remove the bottles and add hot water up to the necks, after which whirl them again for 1 minute.   Again add hot water until the fat rises nearly to the top of the graduations. Whirl again for 1 minute.   Then measure the length of the column of fat by a pair of dividers, the points being placed at the extreme limits of the column, the fat being kept warm, if necessary by standing the bottles in water at 60°C.   If now one point of the dividers is placed at the 0 mark of the scale on the bottle used, the other will indicate the percentage of fat in the milk.

Notes.—Methods based on centrifugal separation of the fat, of which the Babcock method is the pioneer, are by far the most rapid and convenient for general use.   They have practically replaced the more tedious extraction methods and are universally employed in creameries and milk depots.

When the acid and milk are mixed the mixture becomes hot and turns dark-colored on account of the charring of the milk sugar.   The casein is first precipitated and then dissolved.   The retarding effect of the milk-serum solids being thus eliminated, the fat globules are free to collect in a mass.

The fat obtained should be of a clear, golden-yellow color, and distinctly separated from the acid solution beneath it. If the fat is light-colored or whitish, often with a layer of white particles beneath it, it generally indicates that the acid is too weak or that the milk was too cold when the acid was added. A dark-colored fat with a substratum of black particles indicates that the acid is too strong.   The best results will be obtained by the use of acid of the strength noted above.

The capacity of the graduated neck of the bottle between the 0 and 10 marks is 2 cc.   The specific gravity of warm milk fat is 0.9; hence 2 cc. will weigh 1.8 grams or one-tenth of the weight of 17.6 cc. milk (approximately 18 grams).   The measurement to the extreme limits of the column of fat, rather than to the upper meniscus, is to correct for the small amount of fat, 0.1 to 0.2 per cent, that remains in the acid solution.

Milk that has been preserved with formaldehyde usually requires a longer time and more vigorous shaking to dissolve the curd, on account of the hardening action of this preservative

on the coagulated casein. It is often advantageous to stand the bottles in water at 60°C. for a time before whirling. Samples containing formaldehyde will usually give a violet color when the acid is added to the milk (see page 102).

The Babcock method is so commonly employed for the commercial valuation of milk on a basis of its fat content and to serve as a basis for legal action that it has been found advisable to standardize carefully all details of the procedure and to employ only glassware meeting rigid specifications. In cases of controversy, therefore, the official[1] method should be followed closely.

2. *Röse-Gottlieb Method.*[2]—*a. Using Ordinary Laboratory Apparatus.*—With a pipette place 5 cc. of milk in a 50-cc. glass-stoppered cylinder and add the following reagents, being careful to add them in the order given and to shake the stoppered cylinder thoroughly after the addition of each reagent: 1 cc. of ammonia (sp. gr. 0.96), 5 cc. of alcohol, 12.5 cc. of ethyl ether, and 12.5 cc. of petroleum ether. Let the cylinder stand until the lower layer is free from bubbles, several hours if necessary. Transfer the upper layer to a weighed flask by means of an arrangement similar to a wash bottle as shown in Fig. 43. Adjust the sliding tube until the end rests just above the junction of the two layers, then, by gently blowing, force out the upper layer into the flask. Repeat the extraction, using 10 cc. each of ethyl ether and petroleum ether, and blowing it off into the flask as before. Distill off the solvent and dry the residual fat to constant weight at 100°C. Dissolve the weighed fat in a little petroleum ether. If a residue is found, due to a trace of the aqueous layer which was blown off with the mixed ethers, wash it several times in the flask by careful decantation with petroleum ether. Finally dry and weigh the flask and residue, and deduct from the previous weight. The difference is the weight of purified fat.
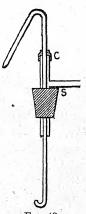
Fig. 43.

b. Using Special Apparatus.—More convenient than the apparatus described above is the Röhrig tube, shown without its base in Fig. 44. The method is practically the same as before,

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[1] *Assoc. Off. Agr. Chem.,* "Official Methods of Analysis," **1935**, p. 268.
[2] Röse: *Z. angew. Chem.,* **1888**, 100; Gottlieb: *Landw. Vers. Sta.,* **1892**, 6.

except that the quantity of milk and of the added reagents is usually doubled. The separation of the upper ether-fat layer is more easily accomplished by means of the stopcock.

The most convenient apparatus of all for this test, in the writer's opinion, is the Mojonnier flask or tube shown in Fig. 45. It consists of two bulbs, a large mixing bulb and a smaller settling bulb at an angle, the two being joined by a constriction.

Method.—Pipette 10 cc. of milk into the Mojonnier flask and add 1.5 cc. of strong ammonia. Mix in the small bulb of the flask. Add 10 cc. of alcohol, insert a soft, well-fitting cork and shake thoroughly, holding the flask horizontally, with the large bulb below, and holding the cork firmly in place with the finger. Add 25 cc. of ethyl ether and shake as before for 20 seconds. Add finally 25 cc. of petroleum ether, shake for 20 seconds, and stand the flask upright (in a beaker or box) for 5 minutes.

Remove the cork by twisting it carefully from the flask. Pour off the ether layer into a weighed beaker as thoroughly as possible, holding the flask horizontally, large bulb uppermost, and looking down upon the flask to watch the dividing line

Fig. 44.—Röhrig tube.   Fig. 45.—Mojonnier flask.   (*Courtesy of Mojonnier Bros. Co.*)

between the ether and aqueous solutions. With care the separation can be made to 2 to 3 drops, provided the dividing line is at the upper level of the constriction at the beginning.

Repeat the extraction, adding in turn, with thorough shaking after each addition, 5 cc. of alcohol, 15 cc. of ethyl ether and 15 cc. of petroleum ether. Let stand for 5 minutes, add water carefully from a wash bottle, if necessary, to raise the dividing line of the ether to the upper level of the constriction, and pour off the ether as before. Distill off the solvent and dry and purify the fat as directed in (*a*).

Notes.—All the successful methods for determining the fat by direct extraction from the milk itself involve the complete or partial solution of the casein. In the Röse-Gottlieb method the casein, precipitated from the milk in very finely divided form by the alcohol, is dissolved by the ammonia. The fat is dissolved by the ethyl ether, and the addition of petroleum ether is to render less soluble the milk sugar or other non-fatty solids that would be dissolved by ethyl ether alone.

The method, while applicable to whole milk, is especially valuable in determining fat in such products as skim milk or buttermilk, which are low in fat. In such cases it is better to use 10 cc. of milk and double the quantity of reagents.

The method has been described in detail because it is the most generally applicable to all such products as sweetened condensed milk, milk powder, malted milk, and the like, with which ordinary extraction methods are difficult because of the protective casein or sugar.

Milk Sugar.—The sugar in milk can be determined either by means of its optical rotation or by its reducing action on Fehling's solution.

The first of these depends upon the fact that after removal of the protein the lactose is the only optically active substance in the milk. Hence it may be determined from its optical rotation after precipitating the protein, commonly done with acid mercuric nitrate. The volume of precipitate is, however, a somewhat variable quantity, depending upon the composition of the milk; hence the true volume of the sugar solution is an unknown quantity. This may be corrected by an arbitrary factor, giving only approximate results, or by the method of "double dilution," which requires close measurements of rotating power to have any real meaning. Further, the amount of lactose in milk is small for accurate determination by any modern saccharimeter, which requires a normal weight of nearly 33 grams of pure lactose;

while a rotary polariscope would require almost 100 grams. Hence the copper reduction method, at its best with small amounts of sugar, is the only one described here. If desired, details of the optical method will be found in the authorities cited below.[1]

Determination by Fehling's Solution.—Pipette 25 cc. of milk into a 500-cc. graduated flask. Add about 400 cc. of water, 10 cc. of copper sulphate solution,[2] then 35 cc. of $0.1N$ sodium hydroxide (or an equivalent quantity of a stronger solution) and make up to 500 cc. Mix thoroughly and filter through a dry filter. Use 50 cc. of the filtrate for the determination of the lactose, as described on pages 262 to 275. Express the result as per cent of lactose monohydrate, calculating the weight of 25 cc. of the milk from its specific gravity.

Notes.—Before the lactose can be determined by Fehling's solution, the proteins, being copper reducing, must be removed. This is done by precipitation with colloidal cupric hydroxide, which then flocculates, removing the colloidal protein and incidentally the fat. The addition of alkali should be such that a slight excess of copper still remains in solution, since an excess of alkali will prevent the precipitation of part of the protein. The quantity stated in the procedure is correct for most milks.

On account of the considerable dilution of the sample the volume of the precipitated protein and fat need not be considered.

Proteins. Determination of Total Protein.—This is best done by the Kjeldahl method. Weigh 5 grams of milk into a Kjeldahl flask (or measure 5 cc. and calculate the weight from the specific gravity) and carry out the determination as described on page 42.

The tendency of the alkaline solution to froth during the distillation, which is especially noticeable with milk, can be prevented by the addition of a piece of paraffin the size of a pea. Multiply the per cent of nitrogen by the factor 6.38 to obtain the per cent of protein.

Separation of Casein and Albumin.—Casein can be separated from albumin by precipitation with acid at a temperature below the coagulating point of the albumin. The method adopted by

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[1] LEACH-WINTON: "Food Inspection and Analysis"; MAHIN: "Quantitative Analysis"; *Assoc. Off. Agr. Chem.*, "Official Methods."

[2] 69.28 grams per liter. The copper sulphate solution used in the Fehling mixture may be conveniently employed.

the Official Agricultural Chemists[1] is practically that worked out by Van Slyke and Hart.[2]

Casein.—To 10 grams of milk add 90 cc. of water at 40 to 42°C. and then at once 1.5 cc. of 10 per cent (by weight) acetic acid. Stir and let it stand 3 to 5 minutes. Then decant on a filter, wash by decantation twice with cold water, and transfer to the filter. Wash twice on the filter. Determine the nitrogen in the washed precipitate and filter by the Kjeldahl method. Multiply by 6.38 for the casein.

Albumin.—To determine the albumin, neutralize the filtrate with caustic alkali and phenolphthalein, add 0.3 cc. of 10 per cent acetic acid, and heat it on the steam bath until the precipitate settles clear. Filter, wash with cold water, and determine the nitrogen as above. Nitrogen multiplied by 6.38 equals albumin.

Notes.—The above methods are based on the fact that casein is flocculated by dilute acids at a lower temperature than the other proteins. Albumin is flocculated and separated by a more dilute acid but at a much higher temperature. The methods are tedious and require considerable experience to give consistent results. Even then it is probable that exact values are not obtained.

If the most accurate determination of casein is desired it should be precipitated at a definite pH. The successful use of acetic acid depends on the fact that casein is a protein that is insoluble at its iso-electric point, and it is to be separated from two others, albumin and globulin, which are typically soluble, even at their iso-electric points, in salt solutions such as are present in milk. It is fundamentally necessary, therefore, as suggested by Waterman, to precipitate the casein at a pH as near this point as possible, which is about pH 4.6. Methods based on this conception have been described by Waterman[3] and by Moir,[4] and their papers should be consulted for a critical discussion of the subject.

If results of somewhat less accuracy only are needed and it is desired to avoid the tedious nitrogen determinations, the follow-

[1] "Official Methods of Analysis," **1935**, p. 266.
[2] *J. Am. Chem. Soc.*, **1893**, 635; *Am. Chem. J.*, **1903**, 170.
[3] *J. Assoc. Off. Agr. Chem.*, **1927**, 259; **1928**, 298; **1930**, 254.
[4] *Analyst*, **1931**, 2, 73, 147, 228.

ing volumetric method, devised by Van Slyke and Bosworth,[1] requires much less time and skill:

Measure 20 cc. of the well-mixed milk into a 200-cc. graduated flask and add about 80 cc. of water. Add 1 cc. of phenolphthalein solution and 0.1N sodium hydroxide until a faint pink color remains throughout the mixture even after considerable shaking. Avoid an excess of alkali.

To the neutralized diluted sample, which should be at a temperature of 18 to 24°C., add 0.1N acetic acid from a burette in 5-cc. portions, shaking vigorously for a few seconds after each addition. After thus adding 25 cc. and shaking, the mixture is allowed to come to rest. If enough acid has been added, the casein separates promptly in large, white flakes, and on standing a short time the supernatant liquid appears clear, not at all milky. If the addition of 25 cc. of acid is insufficient to separate the casein properly, add 1 cc. more of acid and shake; continue this addition of acid, 1 cc. at a time, until the casein separates promptly and completely upon standing a short time. Note the number of cubic centimeters of acid used.

After the casein is completely precipitated make up the mixture to the 200-cc. mark with water, shake thoroughly and filter through a dry filter. Filtration should be rapid and the filtrate quite clear. If marked turbidity is apparent in the filtrate, a new sample should be taken and the process repeated, using more acid than before. Titrate 100 cc. of the filtrate with 0.1N sodium hydroxide and phenolphthalein to a pink color that remains throughout the solution for 30 seconds. Subtracting the number of cubic centimeters of sodium hydroxide from one-half the cubic centimeters of 0.1N acetic acid added will give the cubic centimeters of acid required to precipitate the casein from 10 cc. of milk. (1 cc. of 0.1N acetic acid = 0.11315 gram of casein.)

Calculation of Milk Solids.—It has long been recognized that in normal milk the constituents are present in a fairly constant ratio. This being true, it should be possible, having determined two factors, to find a third by calculation, or at least to show by such calculation a sufficient variation from the normal to indicate the adulteration of the sample. For example, given the lactometer reading and fat, to calculate the total solids:

[1] *Ind. Eng. Chem.*, **1909**, 768.

L = the lactometer reading.

s = increase in lactometer reading by 1 per cent solids not fat.

f = decrease in lactometer reading by 1 per cent fat.

T = total solids.

S = percentage of solids not fat.

F = percentage of fat.

Then

$$L = Ss - Ff.$$

Since

$$S = T - F.$$
$$L = (T - F)s - Ff,$$

Whence

$$T = \frac{L + Ff}{s} + F.$$

The uncertainty of the calculation lies in the values for s and f, which on account of the difference in solution densities of the components of the solids not fat are not absolute constants.

Based on the principle just stated, various formulas have been proposed for the calculation of milk solids. One of the simplest of these is that of Hehner and Richmond.[1]

$$T = \frac{L}{4} + 1.2F + 0.14,$$

when T is the percentage of total solids, L the reading of the lactometer, and F the fat.

The Babcock[2] formula for solids not fat, largely used in this country, is

$$\text{Solids not fat} = \left(\frac{100S - Sf}{100 - 1.0753\ Sf} - 1 \right) \times (100 - f)\ 2.5,$$

where S is the specific gravity, and f the percentage of fat.

When a number of calculations are to be made, Richmond's "milk scale" (Fig. 46) will be found convenient. This is an instrument based on the principles of the slide rule, having three scales, two of which, for the fat and the total solids, are marked

[1] *Analyst*, **1888**, 129; **1892**, 169; **1895**, 57.

[2] *Wis. Agr. Expt. Sta.*, 12*th Ann. Rept.*, **1895**, 120.

on the body of the rule, while that for the lactometer readings is marked on the sliding part.

A similar relation has been worked out for the proteins, so that if a constant value be assumed for the ash the composition of a sample may be determined with a fair degree of approximation from the two simple determinations of specific gravity and Babcock test.

The relation between the proteins and fat has been expressed by Van Slyke[1] as $P = 0.4 \ (F - 3) + 2.8$. Similarly Olson[2]

Fig. 46.—Richmond's milk scale.

has proposed the following formula for calculating the protein from the total solids ($T.S.$):

$$P = T.S. - \frac{T.S.}{1.34}.$$

These values will naturally be most nearly correct in the case of normal average milk. With watered or skimmed milk they will be only approximate.

In the table below the calculated values on a sample are compared with those actually determined:

Determination	Actual values	Calculated values
Lactometer reading...................	33.0	
Fat (Babcock)......................	3.80	
Total solids........................	12.73	12.95
Ash...............................	0.71	0.7 (assumed)
Proteins...........................	3.33	3.12 (Van Slyke)
		3.29 (Olson)
Milk sugar.........................	5.04	5.16
Solids not fat......................	8.93	9.15

Examination of Milk Serum.—The most variable constituents of normal milk are the fat and proteins, especially the former; the least variable are the ash and milk sugar. The milk serum,

[1] *J. Am. Chem. Soc.*, **1908**, 1182.

[2] *Ind. Eng. Chem.*, **1909**, 253.

or milk from which the fat and proteins have been removed, is therefore of more uniform composition than the milk itself, hence better suited for the detection of adulteration and especially of added water. The serum may be prepared by adding to the milk some suitable precipitant of the proteins, as calcium chloride, acetic acid, or copper sulphate, the fat being carried down by the precipitated proteins. The clear liquid after filtration may be examined for its content of dissolved solids, its specific gravity, or most conveniently by the immersion refractometer. The calcium chloride method[1] is largely employed in Germany, but most of the data available on American milks have been obtained by the use of the other two methods.

The copper sulphate method is preferable to the use of other precipitants in that it is quicker, there is no heating with consequent chance for loss by evaporation, and the variation in results is less in the case of pure milk. Ten per cent of added water will usually show by the decreased refraction, when not less than 15 per cent would be detected by the acetic acid method.

The Copper Sulphate Method.[2]—Dissolve 72.5 grams of crystallized copper sulphate in water and dilute to a liter. This solution should be adjusted, if necessary, so that it will refract at 36 on the scale of the immersion refractometer at 20°C., or have a specific gravity of 1.0443 at 20°C., compared with water at 4°C. To one volume of the copper solution add four volumes of milk, shake well and filter. The filtrate will usually be clear after the first few drops have passed through. On the clear filtrate, either the refraction at 20°C., the specific gravity at $\frac{20°}{4°}$C., or the total solids may be determined.

Examination of the copper serum from 150 samples of known purity milk gave refractions varying from 36.1 to 39.5, while the total solids of the same samples showed a range from 17.37 to 10.40 per cent and the fat varied from 7.7 to 2.45 per cent, which is sufficient evidence of the value of the method.

The minimum values for the copper serum of normal milk are 36 for the refraction at 20°C., 1.0245 for the specific gravity at $\frac{20°}{4°}$C. and 5.28 per cent for total solids.

[1] ACKERMANN: *Z. Nahr.-Genussm.*, **1906**, 405; **1907**, 186.
[2] LYTHGOE: *Mass. State Bd. Health, Ann. Rept.*, **1908**, p. 594.

The copper sulphate method should be used only on fresh milk. If the milk is already soured, it may be filtered and similar determinations made on the natural sour serum, which for unwatered milk should not refract below 38.3 or have a specific gravity at $\frac{20°}{4°}$C. below 1.0229.

The greatest value of the sour serum, however, is for the determination of ash, which may be done by evaporating 25 cc. of it to dryness in a platinum dish and igniting in a muffle at a temperature not above 600°C. In unwatered milk the ash content of the sour serum should not be less than 0.73 per cent. The special value of the determination, as pointed out by Lythgoe,[1] lies in the fact that there is no relation between the refraction of the serum and the ash of the sour serum; hence, if both results are below the minimum for pure milk, it is positive indication of the presence of added water.

If a refractometer is not available, it is possible to calculate the refraction from the density, using the established relationship between the density and refractive index of liquids. The value of the refractive constant, K, for the formula of Lorenz and Lorentz, $\frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d} = K$, where n = the refractive index at 20°C. and d = the density at $\frac{20°}{4°}$C., has been determined by Lythgoe to be 0.20526 for the copper serum and 0.20607 for the sour serum. Having determined, then, the density at $\frac{20°}{4°}$, the refractive index may be calculated by the appropriate constant and the corresponding reading of the immersion refractometer taken from Table 3, page 16.

Cryoscopic Test.—Another physical test of considerable value for the detection of added water is the determination of the freezing point of the milk. The freezing point of normal milk is a physiological constant varying within very narrow limits, depending primarily upon the osmotic pressure of the secretions of the animal. Milk is secreted with a very uniform osmotic pressure. Usually, therefore, the lactose and soluble solids remain practically constant, which, as we have seen, is the fundamental basis for the examination of the milk serum. If the

[1] *Ind. Eng. Chem.,* **1914,** 899.

lactose occasionally falls below the average, the proportion of soluble salts in the milk rises enough to keep the osmotic pressure normal. Since for equal weights the osmotic pressure of salt is much higher than that of sugar, a milk of this kind will have an abnormally low percentage of solids not fat, but, since its osmotic pressure and hence its freezing point remain normal, a determination of the latter will show that the milk is not watered. Conversely, the watering, reducing both the lactose and salts in the same proportion, will show in the altered freezing point, in spite of the variable composition of genuine milk.

Examination of over 1,000 samples of English milk by Elsdon and Stubbs[1] showed a variation for the freezing-point depression from 0.529 to 0.563°C., with an average of 0.544°C.; extensive tests in this country by Hortvet (*loc. cit.*), and Bailey[2] have shown results between 0.530 and 0.566°C., with an average value of 0.545°C. The freezing point is undoubtedly the most nearly constant of any of the properties exhibited by milk. As milk is diluted with water its freezing point approaches that of water, and depressions less than 0.530°C. will be observed. If fresh milk gives a freezing-point depression of less than 0.53°C., it is generally taken as definite proof of the presence of added water. By comparing the freezing-point depression of milk which has been diluted with water with the minimum or the accepted average depression observed for milk of known purity, the percentage of water added can be closely approximated.

The method requires the use of a special apparatus or cryoscope for the accurate determination of minute differences in freezing point. Of the various forms of apparatus devised for this purpose the one that has been most commonly employed in this country and the one adopted by the Association of Official Agricultural Chemists[3] is that proposed by Hortvet.[4] (Fig. 47.) A description of the apparatus and the detailed procedure for its use are not given here because, although it probably possesses unique value in those cases where the evidence of other methods is conflicting or inconclusive, the method is not entirely suited for occasional use since it involves the handling of a considerable

[1] *Analyst*, **1934**, 149.
[2] *J. Assoc. Off. Agr. Chem.*, **1922**, 484.
[3] "Official Methods of Analysis," **1935**, p. 270.
[4] *Ind. Eng. Chem.*, **1921**, 198.

quantity of ether and requires a certain amount of skill and practice in manipulation to obtain good results. Once this experience has been gained, however, the method is reasonably rapid in its operation and affords without serious difficulty information hardly to be obtained in any other way.

Full details of the construction, standardization, and use of the apparatus may be found in the references cited.[1] A critical discussion of the technique of the method and the principles involved will be found also in the excellent papers of Elsdon and Stubbs.[2] Note that freezing-point determinations should be made in general only on samples of milk which are fairly sweet or fresh, *i.e.*, showing an acidity not appreciably above 0.18 per cent as lactic acid by the method on page 128. The method is, however, equally good with pasteurized milk.

The method can be used not only to show the presence of added water with a delicacy not attained by other methods, but in many cases a close approximation to the percentage added may be made as well.

Elsdon and Stubbs (*loc. cit.*) have pointed out that the formula for calculating added water as given in the "Official Methods" should be corrected to the customary method of reporting results as per cent by *weight*. They give an improved formula for this.

Fig. 47.—Hortvet cryoscope. (*Courtesy of Arthur H. Thomas Co.*)

Special Tests for Adulterants

Cane Sugar.—Cane sugar may be present in milk from diluted condensed milk used to eke out the supply, or may be present

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[1] For further valuable suggestions on the setting up of the cryoscope see HORTVET: *J. Assoc. Off. Agr. Chem.*, **1923**, 424 *et seq.*

[2] *Analyst*, **1934**, 585; **1935**, 147.

from calcium saccharate, added as a thickening agent (see page 160, under Cream). It is evident that any considerable amount which had been added would be detected by the taste. Its presence may also be shown by the resorcin test described on page 262 or by the reduction of ammonium molybdate, as in testing for calcium saccharate in cream.

Gelatin and Calcium Sucrate.—These substances, although possibly used to thicken milk, are more commonly found in cream and their detection is described on pages 157 and 160.

Preservatives.—In the past such preservatives as formaldehyde, boric acid, and borax, or mixtures of the two latter, and possibly hydrogen peroxide and fluorides have been reported in milk. At present, however, their use is extremely limited, although it may be necessary to make an occasional test for their presence; hence no extended discussion of preservatives is given here.

Formaldehyde.—This is the ideal preservative for milk, being readily used and by far the most efficient. Quantities that give a proportion in the milk of from 1 in 10,000 parts to 1 in 50,000 have been employed. Such an amount will suffice to preserve the milk from 24 hours to several days. Larger quantities, such as 1 part in 3,000, will preserve the milk for months. These large amounts, however, would be more or less apparent by the taste or odor.

Several of the best tests for detecting formaldehyde are described on page 102, in the chapter on Preservatives. These may be applied directly to 10 cc. of the milk; or, as suggested in the gallic acid test, a larger quantity, 25 to 100 cc., may be distilled and the test applied to the first portion of the distillate.

It should be borne in mind that when small amounts of formaldehyde are added to milk the ordinary tests will show the presence of the preservative for only a short time. This is due to the gradual formation of condensation products of the formaldehyde with the proteins of the milk which do not respond to the usual reactions. In such a case it is better to distill the milk as directed and apply the gallic acid test to the distillate.

Another possible contingency is that some substance may be added with the formaldehyde which will interfere with the tests for its detection. Both hydrogen peroxide and nitrites prevent the reaction of formaldehyde in the usual tests, and both of these

have been used in commercial preservatives. The hydrochloric acid-ferric chloride test (page 102) can be used to show the formaldehyde in the presence of considerably larger quantities of nitrite or hydrogen peroxide by previous treatment with urea. Add to 10 cc. of the milk 1 cc. of a 10 per cent solution of urea, then 2 cc. of dilute (1:40) sulphuric acid and immerse the test tube in boiling water for 2 minutes. Cool and carry out the test as usual. The reactions between the urea and the nitrous acid or hydrogen peroxide may be expressed:

$$CO(NH_2)_2 + 2HNO_2 \rightarrow CO_2 + 2N_2 + 3H_2O$$
$$CO(NH_2)_2 + 3H_2O_2 \rightarrow CO_2 + N_2 + 5H_2O.$$

A test with phenylhydrazine hydrochloride and sodium nitroprusside may also be used.[1]

Boric Acid and Borax.—Use from 25 to 100 cc. of the sample and proceed as directed on page 110.

Salicylic and Benzoic Acids.—If it is desired to test for these, the following method may be employed: To 25 cc. of milk add 100 cc. of water and precipitate the proteins and fat with copper sulphate and sodium hydroxide, as described on page 134. Filter and add to the filtrate 5 cc. of concentrated hydrochloric acid. Extract with ether and proceed with the qualitative tests for benzoic and salicylic acids as outlined on page 103 *et seq.*

Fluorides.—To 100 cc. of milk add an equal volume of water, heat to boiling and proceed with the method as given on page 117.

Coloring Matter.—The object in adding coloring matter to milk is in general to disguise the bluish appearance of skimmed or watered milk. For this reason it is rather unusual to find added color in the case of milk that is of standard quality, although such cases have been reported.

Formerly the chief color used was annatto, a reddish-yellow coloring matter obtained from the seeds of *Bixa orellana*, a shrub growing in South America and the West Indies. A solution of the color in very dilute alkali is employed. More recently various coal-tar dyes and even caramel have been used. The latter is perhaps not so likely to be found because its color is too brown and not enough yellow to give the desired creamy appearance to the milk, which is so easily obtained with annatto. The

[1] See *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 437.

coal-tar colors, especially mixtures of yellow and orange azo dyes, give very good results.

If occasion should arise to test milk for color, simple tests will be found described in the "Official Methods" of the Association of Official Agricultural Chemists.

Pasteurized Milk.—The extended use of pasteurized milk, *i.e.*, milk that has in general been kept at 142 to 150°F. for 30 minutes, together with the fact that in many places the sale of raw milk is prohibited unless certified, has brought increasing importance to some means of determining whether or not a given sample of milk has been thus heated.

The various tests that have been proposed for this purpose depend mostly on the partial or complete destruction by heating of certain enzymes present naturally in raw milk. If it is desired to determine simply whether the milk has been heated or not the Schardinger test[1] depending on the destruction of the enzyme *peroxidase* in the pasteurizing process has been much used.

Method.—Mix 20 cc. of milk in a test tube with 1 cc. of a solution containing 5 cc. of a saturated alcoholic solution of methylene blue, 5 cc. of 40 per cent formaldehyde (formalin) and 190 cc. of water. Cover the contents of the tube with a layer of liquid petroleum (as Nujol) to prevent access of air and place the tube in a water bath at a temperature of 45°C. Raw milk will decolorize the reagent in less than 20 minutes, whereas pasteurized milk will take longer.

To distinguish further whether the milk has been improperly pasteurized, *i.e.*, the temperature has been too low or the time too short, or if a small amount of raw milk has been added to help the chemical composition, a more delicate test is needed. For this purpose has been developed the phosphatase test of Kay and Graham.[2] This test depends on the degree of destruction of the enzyme *phosphatase* by heating. The enzyme will liberate phenol under certain definite conditions if raw milk is present or if the milk on which the test is used has been improperly pasteurized. The liberated phenol can be determined by colorimetric

[1] *Z. Nahr.-Genussm.*, **1902**, 1113; NURENBERG and LYTHGOE: U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, 167.

[2] *J. Dairy Research*, **1933**, 63; **1935**, 191; ANDERSON, HERSCHDÖRFER and NEAVE: *Analyst*, **1937**, 86.

tests similar to those employed in water analysis. The details of the method are too long to give here but will be found in the references given, or a good summary can be found in Jacobs, "Chemical Analysis of Foods and Food Products."

INTERPRETATION OF RESULTS

The most common forms of adulteration are still the addition of water and the removal of cream. Reconstituted and homogenized milks have been reported, but this practice is more common with ice cream and cream (which see). By reference to the table on page 124, it will be seen that on account of the variation in the composition of unadulterated cow's milk the detection in all cases is not an easy problem. The variation in the fat content, especially, makes it more difficult to show with certainty the partial removal of cream than the addition of water.

This is well shown in the following table in which A is the normal milk shown on page 124, B the same milk in which the fat has been reduced to 3.6 per cent by adding water, and C the same milk in which the fat has been reduced to 3.6 per cent by skimming.

Determination	A	B	C
Total solids	12.8	11.52	12.39
Fat	4.0	3.60	3.60
Proteins	3.3	2.97	3.06
Sugar	4.8	4.32	4.82
Ash	0.7	0.63	0.71
Solids not fat	8.8	7.92	8.79

It is seen that in sample C it is only the fat that has been decreased to any degree. In fact there is nothing in the figures given for C to indicate in any way that the sample is not genuine milk, while in B the solids not fat are so low as to be distinctly suspicious of watering.

Composition of Milk of Known Purity.—The average composition of milk, together with the usual and the extreme limits of variation, has already been stated on page 124. The greater number of published analyses of genuine cow's milk, a great number of which are available for different countries, have been limited to determinations of solids, fat, and specific gravity.

A summary of a typical series of analyses of samples of known purity is given in Table 12.[1]

A more detailed study, including the constants of the copper serum, will be found in Table 13,[2] which includes the analyses of 33 samples of known-purity milk from individual cows, and 4 samples of herd milk, arranged in the order of their percentage of total solids.

TABLE 12.—SUMMARY OF ANALYSES OF MILK OF KNOWN PURITY

Determination	All samples, (434)			Herd milk, (47)		
	Max., per cent	Min., per cent	Average, per cent	Max., per cent	Min., per cent	Average per cent
Total solids............	17.17	10.20	12.98	14.57	11.56	12.79
Fat...................	7.70	2.45	4.21	5.40	3.35	4.03
Protein..............	5.01	2.00	3.27	4.02	2.66	3.31
Ash..................	0.87	0.56	0.76	0.79	0.65	0.74
Solids not fat..........	10.65	7.50	8.77	9.48	7.63	8.76
Milk sugar............	5.80	3.91	4.78	5.25	4.35	4.83
Protein-fat ratio........	0.99	0.46	0.78	0.95	0.66	0.82
Fat in total solids......	47.4	25.0	32.5	37.1	28.2	31.6
Refraction of copper serum, 20°C.........	40.4	36.0	37.9	38.8	36.7	37.8
Refraction of sour-milk serum, 20°C.........	50.9	38.3	41.9	43.5	41.3	42.3
Ash of sour-milk serum, grams per 100 cc.....	0.932	0.730	0.794	0.852	0.764	0.792

In collecting the samples milk was taken from the heaviest milkers, so as to include a larger proportion of low-grade milk for minimum values. None of the milk could be called exceptionally high grade, as samples were not collected from Jersey or Guernsey cows.

Inspection of this table shows, as would be expected, a great variation in the percentage of fat in the individual samples, the highest being almost 100 per cent higher than the minimum values. The solids not fat are seen to present a much less variation, and, as Lythgoe has pointed out, this variation is due largely to the changes in protein content, the milk sugar and ash remaining fairly constant. Upon this fact depends the special value of the milk serum in showing adulteration.

[1] LYTHGOE: *Ind. Eng. Chem.*, **1914**, 899.
[2] LYTHGOE: *Mass. State Bd. Health Bull.*, **1910**, 422.

Standard Milk.—Nearly all the states have fixed definite standards for milk which may be sold legally, the standard being based usually upon the fat content and the total solids. A list of these will be found in Table 14.

The United States definition[1] is: "*Milk* is the whole, fresh, clean, lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before and 5 days after calving, or such longer period as may be necessary to render the milk practically colostrum free."

The fat and solids standards, formerly stated, are now abandoned for the state and municipal standards, on account of the difficulty in fixing a standard that will apply to all sections.

In some cases all that may be necessary is to show by the analysis that the milk does not conform to the legal standard. In certain of the states, however, a legal distinction is made between milk that is simply below standard and milk that has been actually adulterated by skimming or watering; the latter, implying a willful attempt to defraud, bring the more severe penalties. It is therefore of importance to show by the analysis whether water has been added to the milk directly and not through the breed or feed of the cow.

Detection of Watered Milk.—Since in general the water that has been added is no different from the water already present in the milk, it is evident that this form of adulteration can be detected only by showing chemical or physical changes in the milk that could be ascribed only to the addition of water. Methods have been proposed, it is true, based on differences in the added water, such as an abnormally high amount of nitrates, which might have been derived from the polluted barnyard well, but these methods are of little importance, since water from a pure water supply may equally well be used.

a. Solids Not Fat.—Since the variation in proportion of solids not fat in normal milk is much less than the range of total solids this is of distinct value in showing added water. Although, as indicated in the table of limiting values on page 124, the value for solids not fat may go as low as 7.5 per cent, this is rather uncommon, and a fairer minimum would be 7.7 per cent. A value below 7.7 per cent would certainly be extremely suspicious

[1] U. S. Dept. Agr., *Food Inspection Decision* **200**, August, 1926.

TABLE 13.—ANALYSES OF MILK OF KNOWN PURITY

Breed	Time since calving (mos.)	Weight of milk (lbs.)	Specific gravity 15°	Total solids (per cent.)	Fat (per cent.)	Proteins (per cent.)	Ash (per cent.)	Solids not fat (per cent.)	Sugar (per cent.)	Copper serum				Natural sour serum		
										Refraction 20°	Specific gravity 20°/4°	Solids (per cent.)	Sugar (per cent.)	Refraction 20°	Specific gravity 20°/4°	Ash (per cent.)
Grade Durham	2	15	1.035	14.58	5.10	3.35	0.81	9.48	5.00	39.7	1.0280	6.28	4.43	44.4	1.0292	0.768
Holstein	2	15	1.034	13.65	4.50	3.33	0.72	9.15	4.95	38.4	1.0271	6.05	4.54	43.0	1.0273	0.776
Grade Durham	1	16	1.036	13.52	4.30	3.54	0.81	9.52	5.00	38.9	1.0270	6.09	4.38	.....	.....	.....
Grade	4	20	1.034	13.36	4.30	3.25	0.76	9.06	5.00	38.3	1.0270	5.96	4.63	42.0	1.0279	0.816
Grade	4	18	1.032	13.30	4.40	3.54	0.70	9.30	4.70	38.7	1.0269	6.07	4.56	43.1	1.0280	0.830
Holstein	5	10	1.033	13.29	4.00	3.14	0.78	8.89	5.40	38.8	1.0275	6.10	4.34	42.2	1.0265	0.784
Grade Durham	1	15	1.034	13.27	4.20	3.36	0.66	9.06	5.50	39.7	1.0282	6.20	4.50	.....	.....	.....
Grade Ayrshire	2	12	1.033	13.26	4.35	2.84	0.76	8.85	5.75	39.2	1.0281	6.39	4.78	43.9	1.0286	0.750
Grade Swiss	2	16	1.033	13.20	3.80	3.29	0.69	9.26	5.30	38.6	1.0271	6.05	4.48	40.8	1.0259	0.788
Grade Durham	2	15	1.033	13.20	4.10	3.37	0.80	8.92	5.35	39.6	1.0277	5.86	4.82	42.4	1.0274	0.750
Grade Durham	2	16	1.034	13.02	4.30	3.01	0.76	9.01	5.20	38.3	1.0259	6.19	4.47	43.1	1.0286	0.769
Ayrshire	9	10	1.031	12.91	4.30	3.51	0.68	8.55	5.05	37.3	1.0259	5.73	3.94	.....	.....	.....
Grade Ayrshire	2	21	1.031	12.85	4.00	3.15	0.71	8.78	5.20	38.0	1.0274	5.66	4.40	39.9	1.0254	0.846
Grade Holstein	2	17	1.032	12.78	4.00	2.89	0.69	8.78	4.90	38.1	1.0271	6.07	4.40	42.1	1.0254	0.760
Grade Holstein	2	18	1.032	12.78	3.90	2.97	0.76	8.76	4.35	37.6	1.0268	6.05	4.21	42.2	1.0272	0.768
Holstein	6	10	1.031	12.66	3.80	3.01	0.62	8.84	5.15	38.4	1.0273	5.85	4.71	41.8	1.0269	0.772
Grade Holstein	2	20	1.032	12.64	3.70	3.12	0.74	8.88	5.00	38.6	1.0269	6.11	.....	41.9	1.0266	0.820
Grade Durham	2	18	1.035	12.58	3.50	2.97	0.68	9.04	5.25	38.6	1.0271	5.93	4.14	41.9	1.0270	0.744
Grade Durham	2	18	1.034	12.54	3.80	3.08	0.72	9.00	5.05	37.6	1.0266	6.06	4.14	42.7	1.0271	0.741
Grade Durham	2	15	1.033	12.50	3.80	3.08	0.74	9.08	4.85	37.5	1.0264	6.03	4.38	.....	.....	.....
Holstein	4	10	1.031	12.29	3.40	2.65	0.84	8.49	4.60	37.5	1.0258	5.87	4.11	41.8	1.0264	0.755
Holstein	1	10	1.033	12.27	3.50	3.27	0.74	8.87	4.40	38.6	1.0259	5.82	4.25	41.6	1.0259	0.868
Grade Ayrshire	8	18	1.032	12.12	3.70	3.32	0.78	8.62	4.65	37.2	1.0273	5.75	4.33	40.6	1.0262	0.780
Grade Holstein	8	16	1.032	12.08	3.45	2.89	0.72	8.38	5.00	37.5	1.0271	5.70	4.43	42.1	1.0249	0.804
Grade Holstein	1	20	1.032	12.03	3.10	2.99	0.71	8.58	5.00	36.6	1.0261	6.09	4.61	41.5	1.0270	0.800
Grade Holstein	20	16	1.034	12.03	3.70	2.67	0.71	8.90	4.50	36.3	1.0258	6.07	4.30	39.7	1.0270	0.800
Holstein	16	18	1.030	11.77	3.20	2.48	0.72	8.07	4.30	36.3	1.0250	5.79	3.73	41.0	1.0248	0.740
Holstein	½	15	1.033	11.40	3.15	3.09	0.78	8.20	4.50	36.5	1.0256	5.75	3.88	39.7	1.0258	0.800
Holstein	7	18	1.031	11.21	3.35	2.81	0.80	7.86	4.50	36.3	1.0254	5.47	4.02	38.7	1.0244	0.804
Holstein	10	20	1.031	11.48	3.40	2.78	0.65	8.08	4.00	36.3	1.0259	5.37	3.76	40.6	1.0239	0.792
Grade Holstein	4	16	1.030	10.66	2.85	2.66	0.65	7.81	4.50	36.1	1.0254	5.53	4.22	39.1	1.0255	0.800
Holstein	1	28	1.030	10.20	2.65	2.40	0.73	7.55	4.75	35.8	1.0254	5.51	4.28	38.6	1.0242	0.735
Mixed milk[1]	.....	.....	1.033	13.40	4.20	3.13	0.70	9.20	5.10	38.5	1.0260	6.05	4.09	42.2	1.0272	0.780
Mixed milk[2]	.....	.....	1.033	13.08	4.10	3.23	0.76	8.98	4.65	37.7	1.0262	5.84	4.34	41.4	1.0259	0.788
Mixed milk[3]	.....	.....	1.033	12.73	3.80	3.33	0.71	8.93	4.70	38.0	1.0261	5.94	4.22	41.4	1.0272	0.768
Mixed milk[4]	.....	.....	1.032	12.53	3.70	3.35	0.73	8.83	4.75	37.7	1.0259	5.77	4.28	41.5	1.0261	0.808

[1] Grade Ayrshire, Durham, Shorthorn, and Holstein cows.
[2] Grade Jersey, Ayrshire, and Holstein cows.
[3] Grade Holstein and Grade Jersey cows.
[4] Holstein, Grade Holstein, and Grade Jersey cows.

TABLE 14.—LEGAL STANDARDS FOR MILK, 1930

State	Total solids, per cent	Solids not fat, per cent	Fat, per cent
Arizona..........................	....	8.5	3.25
California......................	11.5	8.5	3.0
Colorado........................	....	....	3.0
Connecticut.....................	11.75	8.5	3.25
Delaware........................	....	8.5	3.25
District of Columbia.............	12.5	9.0	3.5
Florida.........................	11.75	8.5	3.25
Georgia.........................	11.75	8.5	3.25
Idaho...........................	....	8.5	3.25
Illinois........................	11.5	8.5	3.0
Indiana.........................	....	8.5	3.25
Iowa............................	11.5	....	3.0
Kansas..........................	....	....	3.25
Kentucky........................	12.0	8.5	3.25
Louisiana.......................	12.0	8.5	3.5
Maine...........................	11.75	8.5	3.25
Maryland........................	12.5	....	3.5
Massachusetts...................	12.0	....	3.35
Michigan........................	12.0	....	3.0
Minnesota.......................	13.0	....	3.25
Mississippi.....................	11.75	8.5	3.25
Missouri........................	11.75	8.5	3.25
Montana.........................	11.75	8.5	3.25
Nebraska........................	....	....	3.0
Nevada..........................	11.75	8.5	3.25
New Hampshire...................	11.85	....	3.35
New Jersey......................	11.5	....	3.0
New York........................	11.5	....	3.0
North Carolina..................	11.75	8.5	3.25
North Dakota....................	12.0	9.0	3.0
Ohio............................	12.0	9.0	3.0
Oklahoma........................	12.0	8.5	3.5
Oregon..........................	11.7	8.5	3.2
Pennsylvania....................	12.0	....	3.25
Puerto Rico.....................	11.75	8.5	3.25
Rhode Island....................	12.0	....	2.5
South Carolina..................	11.5	8.5	3.0
South Dakota....................	11.75	8.5	3.25
Tennessee.......................	....	8.5	3.5
Texas...........................	....	8.5	3.25
Utah............................	12.0	8.8	3.2
Vermont.........................	11.75	8.5	....
Virginia........................	11.75	8.5	3.25
Washington......................	....	8.5	3.25
West Virginia...................	11.5	8.5	3.0
Wisconsin.......................	....	8.5	3.0
Wyoming.........................	....	8.5	3.25

of added water and if accompanied by correspondingly low values for the constants of the serum could be regarded as direct evidence of adulteration. A better figure would be not the *minimum* but one nearer the value for normal milk, say 8.3 per cent. One thing is certain, however, the legal standard for solids not fat should not be given too great weight, other than to raise a presumption of watering or skimming, for the legal standards are often based on political expediency as well as on scientific knowledge.

b. Milk Serum.—If the preliminary examination indicates a possibility of the samples being watered, an examination of the serum should be made. This may be done by the copper sulphate method, which is described and the minimum values for pure milk stated on page 139. Table 15 shows the effect of systematic watering on the composition of the milk and the constants of the serum in the case of a milk that was above the average in solids not fat and refraction.

It is seen that each 5 per cent of added water lowers the refraction by one scale division; hence with average milk, refracting below 38, 10 per cent of added water could be detected, and with rich milk 15 per cent can usually be found.

Table 15.—Composition of a Sample of Milk Systematically Watered

Added water, per cent.	Solids, per cent.	Fat, per cent.	Solids not fat, per cent.	Copper serum		
				Refraction, 20°	Specific gravity, $\frac{20°}{4°}$	Solids, per cent.
0	13.18	4.20	8.98	38.5	1.0272	6.09
10	11.86	3.78	8.08	36.4	1.0249	5.57
20	10.54	3.36	7.18	34.4	1.0233	5.05
30	9.23	2.94	6.29	32.4	1.0211	4.56
40	7.91	2.52	5.39	30.6	1.0194	4.10
50	6.59	2.10	4.49	28.6	1.0174	

In the particular case given in the table, starting with a rich milk, it is evident that the addition of 10 per cent of water would escape detection.

c. Freezing Point.—In doubtful cases, or as confirmatory evidence, it may be helpful to make the cryoscopic test as men-

tioned on page 140, if the necessary apparatus is available. Definite conclusions should not be drawn, however, unless the apparatus is carefully standardized and a certain amount of facility gained in its use, because trustworthy results are dependent upon strict adherence to such essential conditions as the temperature of the cooling bath, degree of supercooling, and method of using the thermometer. This having been done, the procedure is sufficiently standard to be used as a routine method in milk testing.

In general it may be stated that where the freezing point of the original whole milk is known, results are obtainable to within an error not far from 0.5 per cent, and when the freezing point of the original milk is not known, as with herd milk, the addition of water may safely be reported in an amount as low as 3 per cent. Numerous examples of the values to be expected under American conditions, covering an extremely wide range as to distribution and breeds, will be found in the reports of the referee on dairy products of the Association of Official Agricultural Chemists.[1] References to the work of others have been given on page 141.

Detection of Skimmed Milk.—Watering milk does not in general change the relation of the various constituents to one another, since these are all reduced in the same proportion, but removing the fat does change these ratios. It is immaterial whether the milk is skimmed by the actual removal of some of the fat or whether separator skim milk is added to normal milk. In either case the resulting product will have its fat content largely reduced while the proteins and sugar suffer but little change.

a. Protein-fat Ratio.—A typical fat relationship frequently employed is that of $\frac{protein}{fat}$. In normal milk, especially in the mixed milk of a herd, the percentage of fat is rarely less than the protein (see Table 13, page 149). In 5,500 analyses of American milks compiled by Van Slyke, with a fat content between 3 and 5 per cent, the average amount of fat was 3.92 per cent and the average amount of proteins 3.20 per cent. If such milk be skimmed the fat may be reduced to 1 per cent or

[1] HORTVET: *Ind. Eng. Chem.*, **1921**, 198; *J. Assoc. Off. Agr. Chem.*, **1922**, 477; BAILEY: *J. Assoc. Off. Agr. Chem.*, **1922**, 485; *Conn. Agr. Expt. Sta.*, *Ann Rept.*, **1921, 1922.**

even to 0.1 per cent, but the protein content will still be approximately the same as before. In general, it may be said that a protein-fat ratio approaching 1.0 indicates skimming, the amount being greatest in samples showing the highest ratio. This statement should not be taken to mean, however, that a protein-fat ratio less than 1.0 is necessarily an indication of pure milk. The ratio of 1.0 is undoubtedly a generous standard and, in judging the character of mixed or herd milk, should not be taken literally as a criterion. A better figure to use is 0.90. Average market milk has been found[1] to have a protein-fat ratio of 0.82, and the mixed milk of the Guernsey and Jersey breed of cows has a protein-fat ratio as low as 0.6. Considerable skimmed milk can therefore be added to such milk before the ratio of 0.90 is reached.

Milk representing the mixed milk of many dairies can undoubtedly be declared skimmed from a protein-fat ratio less than 0.90, provided other analytical data are obtained to confirm the conclusion. In several cases of herd milk, where the general character of the milk was known to the analyst through previous analyses, the charge of skimming was successfully maintained in court when the ratio was 0.87. Especially must it be borne in mind that the protein-fat ratio is a function, not only of the breed of cow, but of the solids, the fat, and the refraction of the serum, and must be studied in its relation to these figures. For a valuable study of this kind reference should be made to the paper by Lythgoe already cited.

The milk may with certainty be declared skimmed if the fat falls below 2.2 per cent (the minimum value given in the table on page 124), the solids not fat remaining above the average value of 8.5 per cent. If the fat is above 2.2 per cent and below 3.5 per cent the presence of skimmed milk may be confirmed by making a Kjeldahl nitrogen determination on the suspected sample and calculating the proteins by the factor 6.38. If the protein-fat ratio exceeds the values previously stated, the sample is skimmed. If, however, the fat is above 3.5 per cent this procedure will no longer suffice, since the proteins rarely exceed 3.5 per cent. In these few cases the skimming can be judged only from the high specific gravity, high solids not fat, and correspondingly low fat.

[1] LYTHGOE: *J. Assoc. Off. Agr. Chem.*, **1921**, 16.

b. Specific Gravity of Milk Solids.—The specific gravity of the milk solids is sometimes used to show skimming. Fleischmann's formula for calculating this is

$$x = \frac{TS}{TS - \dfrac{(100 \times Gr) - 100}{Gr}},$$

when TS = the total solids and Gr the specific gravity of the milk.

Example.—A sample of milk contains 12.85 per cent of milk solids and has a specific gravity of 1.031. Required, the specific gravity of the milk solids.

$$x = \frac{12.85}{12.85 - \dfrac{(100 \times 1.031) - 100}{1.031}} = \frac{12.85}{12.85 - 3.006} = 1.306.$$

The specific gravity of the solids of normal milk varies between 1.25 and 1.34. It is not changed by watering the milk but is increased by removing the fat or adding skimmed milk. A value above 1.32 is suspicious, and a specific gravity of the milk solids above 1.40 is regarded as conclusive evidence of skimming.

CREAM

The term *cream* in modern dairy practice is almost invariably applied to the product prepared by centrifugal separators, a method that causes a much more nearly complete separation of the fat from the milk than was possible with the older methods.

Composition.—The following figures illustrate the composition of the cream and skim milk obtained by centrifugal separation of a rather rich sample of milk:

TABLE 16.—COMPOSITION OF MILK, CREAM, AND SKIM MILK

Determination	Milk, per cent.	Skim milk, per cent.	Cream, per cent.
Fat	5.05	0.20	21.95
Total solids	14.10	9.6	26.98
Lactose	4.70	5.05	3.32
Casein	3.50	3.62	2.02
Ash	0.79	0.78	0.58
Specific gravity	1.032	1.034	1.015

The Federal standards for cream require a fat content of not less than 18 per cent; light whipping cream, 30 to 36 per cent fat; heavy cream, more than 36 per cent fat.[1]

Cream has the same constituents that are found in milk, practically the only difference between the two being in the greatly increased fat content and the consequent decrease in the amounts of the other constituents. Typical analyses of both "light" and "heavy" cream are given below:

Variety	Total solids, per cent.	Fat, per cent.	Casein, per cent.	Sugar, per cent.	Ash, per cent.
Heavy cream....	52.77	49.19	....	....	0.26
" "	28.98	21.95	3.02	3.32	0.58
Light cream......	21.07	13.88	2.76	3.75	0.68

Forms of Adulteration.—Since cream is valued for its content of fat, adulteration may consist either in a deficiency of fat or in the substitution of foreign fat for the whole or a part of the butter fat.

The latter form of adulteration is carried out by means of the "homogenizer," or colloid mill, a machine by which the melted fat is introduced into milk or skim milk in such small particles and so intimately mixed that a nearly perfect emulsification results and the product has the appearance of genuine cream. Cream is at times made in this way from butter that has been thoroughly washed and added to skim milk or water and milk powder, the advantage of the process being that the fat can be stored more economically in the form of butter until the period of greatest demand. The term *remade* or *reconstituted* is sometimes used for cream made in this way. Considerable use is found for the homogenized product in the manufacture of ice cream, for which there is an extra demand in hot weather or at holiday seasons. Coconut oil has likewise been reported as being used in cream, as well as oleomargarine.

Substances are also added to cream in order to thicken it, the thickness of the cream being the popular criterion of its richness in fat. If the cream, or the milk itself prior to passage through the separator, has been pasteurized, the product will in many

[1] *Federal Register*, Sept. 26, 1939.

cases be thinner than corresponds to the actual amount of fat present, which is another reason for the use of artificial thickeners. Substances added for this purpose comprise *gelatin, agar-agar,* a substance resembling gelatin in its properties and obtained from a Japanese seaweed, and *sucrate of lime,* sometimes known as "viscogen."

The use of unsweetened condensed milk for thickening cream has also been reported[1] (see also page 159).

Preservatives constitute another form of adulteration, those employed being the same as under Milk.

Methods of Analysis

Fat.—The fat may be determined by the Babcock method as described on page 129, but on account of the thickness of the cream and its high fat content, modifications of the method are advisable. Test bottles of greater capacity and different graduation, sold as "cream bottles," should be used, and the sample of cream taken should be weighed rather than measured. If many determinations are to be made the special "cream balances" sold by dealers in chemical supplies will be found convenient. The bottles are commonly made with suitable graduation for either a 9-gram or 18-gram charge of cream and detailed specifications for the form and graduation are given by the Association of Official Agricultural Chemists.[2]

Directions (For a 9-gram bottle only).—Weigh 9 grams of the thoroughly mixed sample, warmed if necessary, into the test bottle, add 9 cc. of water at 60°C. or above, and mix thoroughly. Add 17.5 cc. of the sulphuric acid and proceed as on page 129, keeping the bottles at 60°C. before taking the final reading.

Note.—If the form of cream bottle with wide neck is used, the meniscus of the fat column will be so considerable as to produce an error in the reading. This may be avoided by adding pure mineral oil, "glymol" or "alboline" which has been colored red or blue with an oil-soluble dye. Add a few drops only of the oil (specific gravity not to exceed 0.85 at 20°C.) to the bottle just before the reading, allowing it to flow down the side of the neck and spread over the fat. The surface separating the fat and the mineral oil is taken as the upper limit of the column.

[1] Lythgoe: *Mass. State Bd. Health, Ann. Rept.,* **1911,** 430.
[2] "Official Methods of Analysis," **1935,** p. 278.

The fat layer should be of a clear yellow or amber color and free from suspended particles. All tests in which the fat column is milky or shows curd or charred matter or gives an indistinct reading should be rejected.

Röse-Gottlieb Method.—Weigh out 2 grams of the cream and carry out the determination as under Milk, page 131.

Total Solids.—Weigh 2 grams into a tared, flat-bottomed platinum dish, add about 5 cc. of water and finish the determination as on page 129.

Lactose, proteins, and ash are determined as previously described under Milk, pages 129 to 136, using preferably aliquot portions of a weighed sample diluted with water to definite volume, such as 25 grams diluted to 100 cc.

Foreign fats may be detected by separating a considerable quantity of the fat in a manner generally similar to that employed in the Röse-Gottlieb process and examining the dried and purified fat by the methods described under Butter, pages 228 to 253.

Preservatives.—Use a diluted sample and follow the methods outlined on pages 143 to 144.

Detection of Thickeners. *Gelatin.*[1]—To about 10 cc. of the cream add about an equal volume of water and mix thoroughly. Then add 10 cc. of acid mercuric nitrate[2] shake, and, after allowing the mixture to stand 5 to 10 minutes, filter. If a considerable amount of gelatin is present the filtrate will be somewhat turbid. To a part of the filtrate add half its volume of a saturated, aqueous solution of picric acid. A yellow precipitate will be produced if gelatin is present.

Note.—Since the acid mercuric nitrate itself will cause a turbidity with picric acid if present in large excess, a positive test should always be confirmed by repeating the test in exactly the same manner with a sample known to be pure, in order to be certain that the precipitate is not due to the reagents. The test will detect about 1 part of gelatin in 10,000 parts of water but is somewhat less delicate with cream.

Fresh milk that has been heated nearly to the boiling point will sometimes give a precipitate under the conditions of the

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[1] STOKES: *Analyst,* **1897,** 320.

[2] Dissolve mercury in twice its weight of nitric acid (sp. gr. 1.42) and add to the solution an equal weight of water. For use in the gelatin test dilute this solution with 10 volumes of water.

test, as will evaporated milk or fresh milk on standing for several hours. The precipitate in these cases, however, is quite flocculent and distinctly different from that obtained with gelatin, which comes down quickly and is very fine, resembling barium sulphate except for its yellow color. In the case of soured, cultured, or fermented milks, the precipitate more closely resembles the gelatin precipitate in appearance, and the test should not be made on other than reasonably fresh samples without making a comparison test on known samples. (See Ferguson and Racicot.[1])

Agar-agar.—Dilute a considerable quantity of cream (50 cc.) with twice its volume of water, add 5 cc. of 10 per cent calcium chloride, and heat in the water bath until the precipitate settles. Filter clear while still hot, cool the filtrate, and add two-thirds its
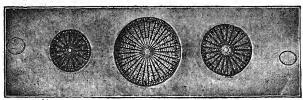
FIG. 48.—*Arachnoidiscus Ehrenbergii* × 100. The smaller oval diatoms are *Cocconeis.* (*Leffman and Beam.*)

volume of 95 per cent alcohol. Filter off the precipitated agar and boil it a few minutes with a small quantity of water, filter hot, and evaporate the filtrate on the water bath to 5 cc. If an appreciable amount of agar is present the solution will gelatinize on cooling.

The presence of agar may often be confirmed by transferring the jelly or the concentrated solution obtained in the test just described to a Kjeldahl flask (see page 38) and destroying the organic matter by heating with sulphuric and nitric acids. The material should be heated with strong sulphuric acid until fumes are given off and then several portions of strong nitric acid (sp. gr. 1.42) added at intervals. When the solution is nearly colorless, dilute and wash several times with water by decantation, or preferably by using a centrifuge. Transfer a drop of the sediment to a microscope slide, cover with a cover glass, and examine under the microscope. Agar-agar frequently, although not

[1] *J. Assoc. Off. Agr. Chem.*, **1936**, 476.

always, contains numerous diatoms, of which the more common are species of *Arachnoidiscus* and *Cocconeis* (Fig. 48).[1]

Homogenized Cream.—This can often be recognized by examination with the microscope, the effect of the process being to render the fat globules much more uniform in size, as well as smaller than in raw cream. Waters[2] found as a result of 100 measurements of raw cream that the average diameter of the fat globules was 2.44μ. The same number of measurements carried out on a homogenized sample gave an average diameter of 0.66μ. A characteristic difference also noticed was the greater uniformity in size of the globules of homogenized cream. The globules of the raw cream varied in size by as much as 6 or 8μ, while in the homogenized cream they rarely differed in size more than 1μ. Another test is to dilute the cream until it has about the fat content of milk, transfer 25 cc. to a small centrifuge tube, and centrifuge at about 1,200 r.p.m. for 2 to 3 minutes. A test should be carried out at the same time on cream known to be normal. The separation of cream (cream line) from the diluted fresh sample will ordinarily be 10 or more times as great as with the homogenized cream.

Reconstituted Cream.—A centrifugal test somewhat similar to the one described above may be used to indicate reconstituted cream.[3] Five cubic centimeters of cream are vigorously shaken with an equal volume of acetone and centrifuged for a minute or so, running a blank test on genuine cream. Natural cream gives no clean separation; whereas cream made from fat and fresh skimmed milk shows an extensive clear layer. If dried skimmed milk has been used there are usually at least three layers formed, a small clear amber-colored upper layer, an opaque middle layer, and a clear liquid with sometimes a packed sediment below.

Condensed Milk.—The use of the sweetened product in any quantity would be shown by a pronounced test for sucrose (see below). The presence of the unsweetened evaporated milk would be indicated by the increased proportion of non-fatty solids and the abnormally high refraction of the serum. This is

[1] For a valuable detailed description of the microscopical appearance of agar see KING: *Analyst*, **1925**, 380.

[2] Thesis, Mass. Inst. Technol., 1910.

[3] RICHARDSON: *Analyst*, **1933**, 686.

shown by the following analysis, in which the first sample is a pure cream and the others are adulterated in this manner.

	Fat, per cent.	Protein, per cent.	Lactose, per cent.	Ash, per cent.	CaO, per cent.	Refraction of copper serum
(a)	38.8	2.20	2.80	0.48	0.106	37.5
(b)	27.8	3.08	3.96	0.66	0.166	42.9
(c)	37.4	3.05	3.94	0.66	0.152	....

Sucrate of Lime (Viscogen).—*a.* In the case of fresh cream the addition of *viscogen* may be indicated in routine examinations simply by the titrable acidity and the pH value.[1] Viscogen being decidedly alkaline, the first of these will be unusually low and the second increased. Bromophenol blue serves well as a qualitative pH test, becoming yellow in fresh cream, yellowish green, greenish blue, or even blue in cream to which viscogen has been added, according to the quantity. A safe precaution in all such tests is to make a direct comparison with cream known to be pure. Other tests commonly used are qualitative tests for sucrose, or somewhat less satisfactory, the increase in the calcium content of the cream.

b. Baier and Neumann's[2] test for sucrose is as follows:

To 25 cc. of cream add 25 cc. of water, 10 cc. of 5 per cent uranyl acetate solution, shake well, allow it to stand for 5 minutes, and filter. To 10 cc. of the clear filtrate add a mixture of 2 cc. of saturated ammonium molybdate solution and 8 cc. of dilute hydrochloric acid (1 volume of acid of 1.12 sp. gr. to 7 volumes of water), and place in a water bath at 80°C. for 5 minutes. If sucrose is present the solution will be of a prussian blue color, which should be compared with the standard prussian blue solution. This is prepared by adding 2 drops of normal potassium ferrocyanide and 5 drops of 10 per cent sulphuric acid to 20 cc. of water containing 1 cc. of a 0.1 per cent ferric chloride solution.

Notes.—The color is due to reduction of the molybdic acid and is not characteristic of sucrose.

[1] PYNE: *Analyst*, **1930**, 747.

[2] *Z. Nahr.-Genussm.*, **1908**, 51; LYTHGOE: U. S. Dept. Agr., *Bur. Chem. Bull.* **122**, p. 52; **132**, p. 122; *Conn. Agr. Expt. Sta., Ann. Rept.*, **1934**, p. 508.

Occasionally a sample of pure milk will be found which will give the test, but to a much less degree than the standard. Moreover the color in this case can be removed by filtration, leaving a green filtrate, while the color due to sugar is not thus removed.

Stannous chloride, ferrous sulphate, and hydrogen sulphide cause a similar reduction in the cold, but will not interfere with the test unless present in such quantities as to change the character of the sample. If no blue color appears before heating, these substances are absent.

c. The Resorcinol Test, page 262, may also be used on the serum prepared with uranyl acetate as described above.

To 3 cc. of the uranyl acetate filtrate add 0.1 gram of resorcinol and 0.3 cc. of 3N hydrochloric acid. Place 0.5 cc. of this mixture in a depression in a porcelain spot plate and allow it to stand at room temperature in a desiccator overnight or until dry. A pink color will be produced if sucrose is present.

TABLE 17.—RELATION BETWEEN FAT AND CALCIUM IN CREAM

Fat, per cent.	Maximum CaO, per cent.	Fat, per cent.	Maximum CaO, per cent.	Fat, per cent.	Maximum CaO, per cent.
15	0.181	28	0.149	41	0.118
16	0.178	29	0.146	42	0.115
17	0.175	30	0.144	43	0.113
18	0.173	31	0.141	44	0.110
19	0.171	32	0.139	45	0.108
20	0.169	33	0.137	46	0.106
21	0.166	34	0.134	47	0.103
22	0.164	35	0.132	48	0.100
23	0.161	36	0.129	49	0.098
24	0.158	37	0.127	50	0.096
25	0.156	38	0.124	......	....
26	0.154	39	0.122	......	....
27	0.151	40	0.120	......	....

d. From the Calcium Content.—Weigh 25 grams of cream into a platinum dish, dry several hours in the water oven, and ignite carefully to ash, preferably in a muffle. Boil the residue in the dish with 20 cc. of dilute sulphuric acid, avoiding loss by spattering, neutralize with dilute sodium hydroxide, and finally add a few drops of acetic acid. Filter, add 1 gram of sodium acetate and ammonium oxalate in excess. Boil, filter, and wash with hot

water. Either determine the calcium oxalate by the ordinary gravimetric method, or dissolve it in hot dilute sulphuric acid and titrate while hot with $0.1N$ potassium permanganate. Calculate as CaO.

Note.—The percentage of calcium in cream varies with the amount of fat, the samples containing a higher percentage of fat having less calcium. From the examination of many genuine samples Lythgoe has arranged the table on page 161 giving the maximum amount of calcium oxide permissible in genuine cream. Pure cream as on the market will ordinarily fall quite a little below the maximum values of the table, since this is largely made from milk that has been pasteurized, that process rendering some of the calcium insoluble and removing it from the cream in the separator.

ICE CREAM

Definition.—Ice cream is the "frozen product of cream, milk or skimmed milk, or any combination thereof, or of milk products, with sugar, and with or without the addition of pure gelatine or pure vegetable gums."[1] The legal definition goes further and specifies the minimum percentage of butter fat which shall be present, varying in different states from 8 to 14 per cent. Some states specify also the minimum of milk solids which the ice cream shall contain.

The product, as made commercially, is a rather complex mixture, the ingredients used at various times comprising, among milk products, one or more of the following: cream, unsalted butter or butter oil, liquid milk, evaporated milk, sweetened condensed milk, condensed skimmed milk, dried milk, skimmed milk powder. Sugar, flavoring materials, nuts, and fruit juices, are commonly found, and eggs may be used in certain forms of ice cream.

Stabilizers or binders are used to produce a smoother body and finer texture, such materials comprising gelatin, vegetable gums as tragacanth or locust bean gum, starch, egg albumin, Irish moss, and the like.

Following are several typical examples of commercial ice cream mixes for a product with 10 to 12 per cent of fat:

[1] Massachusetts state standard.

	Pounds on 100-lb. basis		
	1	2	3
Cream (37.5 per cent fat)..................	25.0	24.5	22.0
Milk (3.8 per cent fat).....................	45.0	55.0	....
Skimmed milk............................	.....	....	40.0
Condensed skimmed milk..................	16.0	....	17.0
Skimmed milk powder.....................	.....	4.5	....
Butter (82.0 per cent fat).................	.....	....	5.0
Sugar...................................	14.0	15.5	15.0
Gelatin.................................	0.35	0.5	0.5

It goes almost without saying that in the choice of ingredients as well as in all subsequent handling, the same care and attention to purity and cleanliness is necessary in order to ensure a good product as in any other branch of the dairy industry.

Manufacture.—After the proper amounts of the various ingredients have been placed in a suitable vat the mixture is generally pasteurized at about 150°F. for 30 minutes to kill any pathogenic microorganisms that may be present. Since this process tends to destroy the valuable property of viscosity of the mix, it is generally restored by "aging" for a number of hours at a temperature just above freezing, or more commonly, the viscosity is restored by the use of a colloid mill or "homogenizer." In many cases the mix is allowed to age further for 12 to 48 hours after homogenizing.

After this aging the mix is run into the freezers, where it is mechanically beaten at the same time in order to incorporate a certain amount of air, which imparts to the ice cream the desired texture. In the process the mixture increases in volume, the swell or overrun, as it is termed, varying from 10 to 100 per cent or more of the original mix. The final stage of the freezing takes place after the mix has been discharged from the freezers and is conducted in a cold or "hardening room" at 0°F. or lower.

Overrun.—This, as mentioned above, means the difference in volume between the ice cream mix and the finished product. It is customary in commercial practice to find that 1 gallon of mix finally becomes about 2 gallons of ice cream, the extra volume being obtained by the incorporation of air. It is an accepted fact that a certain amount of overrun is desirable, converting a com-

pact, relatively unpalatable substance into the light but smooth delicacy that the consumer expects. It is equally obvious, since ice cream is sold by volume, that a limit should be set to the amount of air permitted. This is ordinarily done by setting a minimum limit to the total food solids per gallon of legal product.

Interpretation of Results.—Not much discussion is needed regarding the meaning of the results of analyses, since they are obtained primarily in most cases with a view to learning whether the product conforms to some definite legal or statute standard.

A plain ice cream, made in accordance with good commercial practice, might be expected to show upon analysis:

Fat..................................	10–14 per cent
Milk solids not fat......................	9–11 per cent
Cane sugar............................	14–15 per cent
Gelatin...............................	0.3–0.5 per cent
Total solids...........................	35–38 per cent

The suggested Federal standard for ice cream[1] reads: "Ice cream is a frozen product made from cream and sugar with or without a natural flavoring and containing not less than 14 per cent of milk fat. Fruit and nut ice cream must contain not less than 12 per cent milk fat."

This standard of 14 per cent of fat has not been consistently adopted, being regarded as rather high; and many of the state standards, which are the ones actually in force, ice cream being a more or less localized product, are somewhat lower. In England, where no control was exercised by the authorities, numerous instances were reported of "ice cream" with less than 0.15 per cent of fat. The state standards vary from 8 to 14 per cent for the fat content, and certain of the standards, by fixing also a minimum for milk solids or for total food solids, take into consideration the necessity for control of excessive overrun.

A typical state standard[2] reads:

Ice cream is the frozen product of cream, milk or skimmed milk, or any combination thereof, or of milk products, with sugar, and with or without the addition of pure gelatine or vegetable gums. Such product shall contain not less than ten per cent of milk fat and not less than eighteen and five tenths per cent of total milk solids; provided, that if eggs, fruit, fruit juices, cocoa, chocolate or nuts are added thereto, such

[1] U. S. Dept. Agr., *Office of the Secretary, Circ.* 19.

[2] Massachusetts.

product shall contain not less than eight per cent of milk fat and not less than sixteen and five tenths per cent of total milk solids.

In other states the fat standard is given and also the proviso that the total food solids shall not be less than a fixed minimum, commonly 1.6 lb. per gallon of ice cream. This standard corresponds in effect to a total weight of about 4.6 lb. per gallon and represents approximately an overrun of about 100 per cent for an ice cream with about 35 per cent of total solids. In some cases the weight per gallon of the ice cream is definitely fixed, as at a minimum of 4.5 lb. in New Hampshire. All these limitations are designed to prevent excessive overrun.

METHODS OF ANALYSIS

Preparation of Sample.—In order to keep the sample homogeneous it is better to allow it to melt at room temperature rather than to heat it. Mix thoroughly by stirring or by pouring back and forth from one vessel to another. If the sample contains nuts or fruits it may be passed repeatedly through an ordinary food grinder until the whole material is homogeneous. If the ice cream has separated so much that a representative sample cannot be obtained, the whole sample may be passed through a hand homogenizer.

Fat. Röse-Gottlieb Method.—Weigh accurately by difference 4 to 5 grams of the ice cream into a Mojonnier flask or a Röhrig tube (page 132), add 5 cc. of water, 2 cc. of ammonium hydroxide (sp. gr. 0.90), heat in a water bath to 60°C., stir thoroughly and proceed as directed under Milk, page 132, using, however, the larger quantities of reagents prescribed there (10. cc. of alcohol, 25 cc. each of ethyl ether and petroleum ether).

Notes.—The Röse-Gottlieb method is purposely the only one described for determining fat in ice cream. The Babcock method, as used for liquid milk, is entirely unsatisfactory on account of the heavy char produced by the sulphuric acid and the sugar present. Many modifications of the Babcock test have been proposed, recourse being had to acetic acid, butyl or amyl alcohol, and other reagents to assist in the separation of the fat in a clear column, and even the substitution of various salts in an alkaline medium for the sulphuric acid. They are in general useful only for rough or preliminary determinations of

the fat and even then only when the operator has become entirely familiar with the suggested procedure. For occasional or accurate determinations the Röse-Gottlieb or some similar procedure is the only satisfactory method. The Gerber process, a centrifugal method somewhat similar to the Babcock, is said to give good results, but the apparatus is not in general carried by dealers in this country. Details of various proposed modifications of the Babcock process and the experience of others with them may be found in the references cited below.[1]

Total Solids.—Weigh 2 grams into a tared, flat-bottomed dish, add 5 to 10 cc. of water, and carry out the determination as directed for Milk, page 128.

Note.—If the ice cream is in a container of accepted volume or the actual volume is determined, the value for "total food solids per gallon" may be readily calculated.

Lactose, proteins, and ash are determined as previously described under Milk, pages 129 to 136, using preferably suitable aliquot portions of a weighed sample diluted to a definite volume, say 25 grams diluted to 100 cc. In determining the lactose, use the proper column in the table to correct for the sucrose present.

Sucrose. *Mercury Reagent.*—To 22 grams of yellow mercuric oxide add 30 to 40 cc. of water and sufficient concentrated nitric acid (about 14 cc.) to form a clear solution. Use the least possible excess of the acid. Dilute to about 85 cc. and add 10 per cent sodium hydroxide solution, slowly and with constant shaking, until a slight permanent precipitate is produced. Dilute to 100 cc. and filter. It is best made up in small quantities because if kept for any great length of time the solution tends to become acid, owing to hydrolysis, with the accompanying deposition of basic mercuric nitrate. If this should be the case, add sodium hydroxide solution again to slight permanent precipitation and filter before using.

Determination.—Measure 50 cc. of a dilution of the ice cream, prepared as suggested above, into a 100-cc. volumetric flask, add 25 cc. of water, mix, add 5 cc. of the mercuric nitrate solution and shake thoroughly. Without delay and while shaking or stirring constantly, add enough 2 per cent sodium hydroxide

[1] FRARY: *J. Assoc. Off. Agr. Chem.*, **1934,** 353; CROWE: *Neb. Expt. Sta. Bull.* **246** (1930); OVERMAN and GARRETT: *Ill. Expt. Sta. Bull.* **360** (1930); KNIASEFF: *Ice Cream Trade J.*, **30** (December, 1934); SELBY and SELBY: *Ind. Eng. Chem., Anal. Ed.*, **1939,** 393.

solution to make the sugar solution neutral to litmus paper, taking care to avoid an alkaline reaction. Dilute to the mark, mix thoroughly, and filter through a dry filter paper, rejecting the first 10 cc. of the filtrate.

Polarize the filtrate in a 200-mm. tube, then invert at room temperature, as described on page 293, and polarize the inverted solution as directed on page 290. Correct both the direct and the invert readings for the volumes occupied by the precipitated protein and fat, as determined previously, assuming a volume of 0.8 cc. and 1.075 cc. for 1 gram of protein and fat, respectively. From these corrected readings calculate the sucrose by Clerget's formula as on page 296, correcting further for the weight of sample taken, as compared to the normal weight for the instrument.

Notes.—The method described is practically that of the Association of Official Agricultural Chemists for the determination of sucrose in sweetened condensed milk.[1]

The precipitation of the proteins and fat by the mercuric nitrate is probably a colloidal flocculation, as with the cupric hydroxide used on page 134. The volume of solution in the 100-cc. volumetric flask is less than 100 cc. on account of the volume occupied by the precipitated protein and fat. Hence the value for sugar from the polarization would be too high unless a correction were made. The method of double dilution, in which two flasks of differing volumes are used in the same polarization, is sometimes employed here but is objectionable because of the multiplication of error in reading the dilute solution.

Basic lead acetate, the clarifier ordinarily used in sugar polarizations, is not suitable in the case of milk products, since it has been shown by Wiley[2] that it leaves notable quantities of levorotatory proteins still in solution. The precipitation of proteins by mercuric nitrate, although better, is still not quite complete, Richmond having pointed out[3] that further precipitation can be obtained by the subsequent addition of phosphotungstic acid.

Total Milk Solids.—These may usually be calculated with sufficient accuracy by subtracting the percentage of sucrose from the total solids.

[1] "Official Methods," **1935**, p. 281.

[2] *Am. Chem. J.*, **1884**, 289.

[3] *Analyst*, **1910**, 517.

Foreign fats, of which the most commonly reported have been oleomargarine and coconut oil, may be detected as described under Cream, page 157.

Stabilizers, Gums and Thickeners.—*Gelatin.* See under Cream, page 157.

Vegetable gums.—The presence of gums may be shown qualitatively by removing the proteins with a suitable precipitant, leaving the gums in solution. These are then precipitated by alcohol and identified by their physical properties and by partial conversion to pentoses.[1]

Procedure.—To about 25 grams of the sample add 20 cc. of boiling water, 4 or 5 drops of strong ammonia, mix thoroughly, and transfer to a suitable centrifuge bottle or tube. Neutralize with concentrated acetic acid, using litmus paper as indicator, and add 4 or 5 drops in excess. Shake thoroughly and centrifuge 10 to 20 minutes. Decant the clear solution, evaporate to half its volume or until a precipitate begins to form, add 5 cc. of trichloracetic acid solution (50 grams in 100 cc.) and keep at about 70°C. until the precipitated milk proteins coagulate. Avoid prolonged heating, which will tend to hydrolyze the gums. Cool and filter clear.

Add 4 volumes of alcohol to the filtrate and allow the mixture to stand for several hours, if necessary, to coagulate the separated gum. A precipitate at this point indicates gums.

Centrifuge the mixture, decant the supernatant liquid, and wash with 70 per cent alcohol. Centrifuge, and again decant or filter. Dissolve the residue in a minimum amount of hot water and reprecipitate with 4 volumes of alcohol containing 2 or 3 drops of acetic acid. This reprecipitation removes traces of sugars retained in the first precipitation.

Allow the precipitate to coagulate, centrifuge, and decant. Dissolve the residue in the smallest amount of water, transfer it to a 50-cc. beaker and evaporate to 5 cc. Add an equal volume of concentrated hydrochloric acid, boil 30 to 60 seconds, and add several crystals of phloroglucinol. A deep amber or cherry-red color confirms the presence of gums.

Notes.—The pentose sugars are especially characterized by the varied color reactions that they give with polyvalent phenols,

[1] Cook and Woodman: *Ind. Eng. Chem.*, **1918**, 530; Cross: *J. Assoc. Off. Agr. Chem.*, **1935**, 432; Hart: *J. Assoc. Off. Agr. Chem.*, **1937**, 527.

as phloroglucinol or orcinol, in the presence of concentrated hydrochloric acid. Hence the test is used for the presence of gums, composed in large part of pentosans, as araban and xylan. Further heating with the acid converts the pentose largely to furfural (see page 261).

In the case of fruit ice creams the pectin present may cause some trouble with the test. Cook and Woodman[1] give directions for its removal. A further detailed discussion of gums in food products with methods for their detection and separation will be found in Jacobs, "Chemical Analysis of Foods and Food Products," page 277.

Selected References

ALLEN: "Commercial Organic Analysis," Vol. IX, 5th ed., Blakiston (1933).

DAVIES: "Chemistry of Milk," Van Nostrand (1936).

ECKLES, COMBS, and MACY: "Milk and Milk Products," McGraw-Hill (1936).

FARRINGTON and WOLL: "Testing Milk and Its Products," Mendota Book Co. (1898).

JACOBS: "Chemical Analysis of Foods and Food Products," Van Nostrand (1938).

PRESCOTT and PROCTOR: "Food Technology," McGraw-Hill (1937).

RACE: "The Examination of Milk," Wiley (1917).

ROGERS: "Fundamentals of Dairy Science," 2d ed., Reinhold (1935).

VAN SLYKE: "Modern Methods of Testing Milk and Milk Products," 3d ed., Judd (1927).

[1] *Loc. cit.*

CHAPTER V

EDIBLE FATS AND OILS

Composition.—The fats and oils, excluding from the term *oil* the mineral and essential oils, are mixtures of glyceryl esters of the fatty acids, a particular oil usually containing a number of these esters of various acids. There is no difference in general composition between the fats and the oils, the latter name being given to those fats that are liquid at ordinary temperatures, the term being sometimes actually a misnomer, since coconut and palm-nut "oils" are really vegetable fats.

General Properties.—When pure, the oils and fats are free from color, odor, and taste, their customary appearance in this respect being due to impurities that have not been removed. They are almost completely insoluble in water and, with the exception of castor oil, nearly so in cold alcohol. Hot alcohol dissolves small quantities, and practically all the oils and fats dissolve readily in chloroform, ether, carbon bisulphide, carbon tetrachloride, and petroleum ether.

When heated, little change is produced, except in the case of the drying oils, until decomposition occurs beyond 250°C., with the formation of the intensely irritating *acrolein*. When acted on by the oxygen of the air, especially in the presence of light and moisture, free fatty acids are liberated and altered with the accompanying production of various aldehydes and acids of lower molecular weight having a disagreeable odor and acrid taste, the fat or oil then being termed "rancid."

The chief properties of the fatty acids more commonly occurring in edible fats and oils[1] are tabulated on page 171.

The acids of the acetic series are all saturated monobasic acids of which the first four alone can be distilled at atmospheric pressure without decomposition. By reference to the table it will be observed that as the molecular weight of the acids increases

[1] LEACH-WINTON: "Food Inspection and Analysis," 4th ed.; ALLEN's "Commercial Organic Analysis," 5th ed., Vol. II.

TABLE 18.—PROPERTIES OF THE FATTY ACIDS

Name	Formula	Melting point C.	Boiling point C.	Sp. gr. 20°C.	Occurs prominently in
		Acetic Series $C_nH_{2n}O_2$			
Butyric........	$C_4H_8O_2$	−6.5°	162.3°	0.959	Butter
Caproic........	$C_6H_{12}O_2$	....	200.0°	0.924	Butter
Caprylic........	$C_8H_{16}O_2$	16.5°	236.0°	0.910	Butter, coconut and palm-nut oils
Capric.........	$C_{10}H_{20}O_2$	31.3°	269.0°	......	Butter, coconut and palm-nut oils
Lauric.........	$C_{12}H_{24}O_2$	43.6°	....	0.883	Coconut oil, palm-nut oil
Myristic........	$C_{14}H_{28}O_2$	53.8°	....	0.858 (60°)	Coconut oil, nutmeg, lard
Palmitic........	$C_{16}H_{32}O_2$	62.6°	....	0.85 (60°)	Nearly all fats and oils
Stearic.........	$C_{18}H_{36}O_2$	69.3°	....	......	Most fats
Arachidic.......	$C_{20}H_{40}O_2$	77.0°	....	......	Peanut oil
Lignoceric......	$C_{24}H_{48}O_2$	80.5°	....	......	Peanut oil
		Oleic Series $C_nH_{2n-2}O_2$			
Hypogaeic......	$C_{16}H_{30}O_2$	33.0°	....	......	Peanut oil
Oleic..........	$C_{18}H_{34}O_2$	14.0°	....	......	Most fats and oils
Erucic.........	$C_{22}H_{42}O_2$	33°–34°	....	......	Rape and mustard oils
		Linoleic Series $C_nH_{2n-4}O_2$			
Linoleic........	$C_{18}H_{32}O_2$	Below 18°	....	......	Drying and semi-drying oils
		Linolenic Series $C_nH_{2n-6}O_2$			
Linolenic.......	$C_{18}H_{30}O_2$	....	....	......	Linseed and other drying oils
		Clupanodonic Series $C_nH_{2n-8}O_2$			
Clupanodonic...	$C_{18}H_{28}O_2$	....	....	......	Whale, cod-liver, and fish oils

there is a corresponding rise in the melting and boiling points while the reverse is true of the specific gravity and the solubility of the acids in water and in cold alcohol. In a general way the same is true of their compounds, the glyceryl esters of the acids, which occur in the fats.

In distinction from these, the acids of the oleic series are unsaturated, as seen in the structural formula for oleic acid below.

$$CH_3 - (CH_2)_7 - CH = CH - (CH_2)_7 - COOH.$$

It is therefore possible to add 2 atoms of halogen to form a saturated compound. The great value of this characteristic from an analytical standpoint is seen in the important determination of the iodine value on page 184. It will be observed from the table that the acids of this series have lower melting points than the corresponding acids of the acetic series; hence they are found more largely in the liquid oils than in the solid fats.

A peculiar characteristic of the acids of the oleic series is that by the action of nitrous acid they are changed to solid isomers. Thus oleic acid, $CH_3 - (CH_2)_7 - CH = CH - (CH_2)_7 - COOH$, with a melting point of 14°, is changed to the isomeric crystalline *elaidic acid*, which melts at 51°. The so-called "elaidin test," dependent upon this property, will be found described in standard works on oil analysis, but for the general examination of edible oils affords no information that is not given by other and more reliable methods. Still other iso-oleic acids, as well as isomers of solid acids, are formed in the hydrogenation of oils.

Another property of the unsaturated acids which is of analytical importance is the solubility of their lead salts in ether. If a mixture of saturated and unsaturated fatty acids be precipitated with lead acetate and the insoluble lead soaps thus formed be allowed to stand with ether, the lead oleate, hypogeate, and linoleate will be dissolved, leaving the salts of the saturated acids. This method is used in the Renard test for peanut oil (page 207) to separate the arachidic acid and facilitate its crystallization.

Linoleic acid, the important constituent of the "drying oils," resembles the unsaturated acids of the previous group, but is capable of absorbing 4 atoms of halogen and will also absorb oxygen from the air, becoming thick and viscid. When exposed in a thin layer to air it forms a varnish.

Linolenic and clupanodonic acids are of less interest because they are not so common in edible oils.

Classification.—In any systematic study of the oils it is advantageous to group together those that bear some resemblance in their physical properties or chemical nature. The classification given below, condensed from that given in Allen's "Commercial Organic Analysis," comprises the important edible oils.

a. Olive-oil group (olive, almond, peanut), *vegetable oleins* containing chiefly olein with smaller amounts of the glycerides of palmitic, stearic, arachidic, and in some cases linoleic acids. They are characterized by rather low iodine and saponification numbers.

b. Rape-oil group (rape, mustard-seed oils), fatty oils from seeds of the *Cruciferæ* having distinctly lower saponification values than the oils of the preceding group.

c. Cottonseed-oil group (cottonseed, corn, sesame), *semidrying oils* consisting chiefly of olein and linolein. They have in general fairly high iodine values.

d. Linseed-oil group (poppy, sunflower, soybean), differing from the semidrying oils in the greater proportion of the glycerides of the highly unsaturated acids, linoleic and linolenic. The most important member of the group commercially, linseed oil, need not be included among the edible oils.

e. Cocoa-butter group (cocoa butter, cottonseed stearin), *vegetable fats* comprising chiefly the glycerides of the higher fatty acids as myristic, palmitic, stearic, and oleic. They contain only small amounts of the glycerides of acids below myristic.

f. Coconut-oil group (coconut oil, palm-nut oil), *vegetable fats* distinguished from the preceding group by higher saponification values (showing glycerides of lower fatty acids) and low iodine values (showing the small proportion of unsaturated acids). The larger proportion of lower fatty acids is shown also by their relatively high Reichert-Meissl numbers (see page 199).

g. Lard-oil group (lard oil), *animal oleins*, liquid at ordinary temperatures and consisting mainly of olein. They correspond to the vegetable oils of group *a* although having lower iodine values.

h. Tallow group (butter, beef fat, lard, mutton fat, tallow), *animal fats* which in distinction from the previous group are solid

at ordinary temperatures. They consist chiefly of olein, palmitin, and stearin, with the notable exception of butter, which contains considerable amounts of the glycerides of butyric and other low fatty acids.

METHODS OF ANALYSIS

Object of Oil Analysis.—The term *oil analysis* is somewhat of a misnomer in that as ordinarily carried out it is entirely different from an analysis in which the percentage of the constituents present is determined. The object is usually to determine the purity of a given sample or in the case of a product of unknown source to determine what oil or oils are present. It is therefore customary to determine the "analytical constants" of the oil or mixture, by which is meant those chemical or physical tests that give characteristic values for the different oils, the variation in the "constant" being relatively slight for a particular oil if pure. It will be evident that for this reason testing a known oil for purity will be in general less difficult than determining what oils may be present in an unknown mixture. Even then the difficulty is increased by the fact that the ranges of a given constant for two different oils may overlap, which means that a number of constants may have to be determined. It is apparent also that the differences in these constants for the various oils must be due entirely to differences in the fatty acids which are present, glycerol being common to all.

Sherman[1] has pointed out concisely that the differences to be expected are due mainly: (1) to differences in the mean molecular weight of the acids that are present, or the relative proportion of acids of high and of low molecular weight; and (2) in the relative number of "double bonds," depending upon the proportion of unsaturated acids such as oleic and linoleic, that are present. Of the constants that are described below, typical of the first class would be the saponification number, which is a measure of the molecular weight of the acids present, and the Reichert-Meissl number, which depends upon the proportion of acids of low molecular weight. Of those that depend upon the proportion of unsaturated acids present, the iodine value and Maumené number are typical.

[1] "Organic Analysis," 2d ed., p. 144.

Certain of the constants, as the specific gravity and melting point, are more general, being simply composites of the values due to all the acids present.

The problem is sometimes simplified by the fact that some oils may contain glycerides of rather characteristic acids, as butyric in butter, arachidic in peanut oil, or, as found in commerce, even edible oils may contain definite impurities which within limits will serve to characterize them.

PHYSICAL METHODS

Specific Gravity.—The specific gravity of oils is theoretically determined by the Westphal balance or pyknometer at the standard temperature of 15.5°C., for which temperature most of the older values have been reported. Owing to the high coefficient of expansion of oils the precautions detailed in the chapter on General Methods, pages 1 to 5, should be carefully observed. It is generally found preferable to make the determination at a higher temperature, $\dfrac{20°}{20°}$ or $\dfrac{25°}{25°}$,[1] in which case the result should be corrected by the following formula:

$$Sp_{15.5} = k\, Sp_t,$$

where Sp_t is the specific gravity obtained at $t°$, and k is a factor varying with the temperature. The values of this factor for ordinary temperatures are[2]

16°	1.00035	21°	1.00391
17°	1.00106	22°	1.00462
18°	1.00177	23°	1.00534
19°	1.00248	24°	1.00605
20°	1.00319	25°	1.00677

If it is desired to obtain the specific gravity at a temperature considerably above room temperature the Sprengel tube (page 3) will be found especially suitable, as it may be easily filled with the oil, then suspended in a beaker of water and adjusted when at the desired temperature. If it is then removed and

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[1] This is the standard temperature recommended by the Association of Official Agricultural Chemists, the value for k, the change in specific gravity for 1°C. (page 176) being taken as 0.0007.

[2] WRIGHT: *J. Soc. Chem. Ind.*, **1907, 513.**

cooled to room temperature it can be wiped dry and weighed with no danger of loss of contents.

In the case of fats that are solid at ordinary temperature the determination is best made at a temperature considerably above their melting point, conveniently at 40 or 50°C., the Sprengel tube being previously warmed, then filled with the melted fat, the test being finished as usual. The value may be calculated to 15.5° by the formula

$$Sp_{15.5} = Sp_t + k(t - 15.5),$$

where Sp_t is the value obtained at the temperature t, and k is the change in specific gravity for 1°C. The values for k for the common edible fats are

Cocoa butter	0.000717
Tallow	0.000673
Lard	0.000650
Butter fat	0.000617
Coconut stearin	0.000674
Coconut oil	0.000642
Palm oil	0.000657
Stearic acid	0.000750
Oleic acid	0.000656

Note.—The specific gravity is a constant that does not vary greatly for any given kind of oil, provided it is examined while pure and fresh, but is readily affected by age, rancidity, and any special treatment of the oil.

TABLE 19.—COMMON EDIBLE OILS IN THE ORDER OF THEIR SPECIFIC GRAVITIES[1]

Cocoa butter	0.960	Cottonseed oil	0.922
Beef tallow	0.947	Palm oil	0.922
Mutton tallow	0.944	Cottonseed stearin	0.921
Butter fat	0.936	Peanut oil	0.918
Lard	0.934	Almond oil	0.917
Coconut oil	0.926	Mustard oil	0.917
Poppyseed oil	0.925	Olive oil	0.916
Soybean oil	0.925	Tea-seed oil	0.916
Sunflower oil	0.925	Lard oil	0.915
Corn oil	0.924	Rape oil	0.915
Sesame oil	0.923		

[1] Average values at $\frac{15.5°}{15.5°}$; see Table 27, p. 199, for usual variations.

The different values obtained with different oils are due to differences in the fatty acids present, increasing with increased

molecular weight of the combined acids and with greater percentages of unsaturated and hydroxyl acids. In the case of rancid oils the iodine number and refractive index decrease, the specific gravity, Reichert-Meissl number, Polenske value, acid value, and unsaponifiable matter all tend to increase.

Refractive Index.—The manipulation of the Abbe refractometer and the principle on which it is based have been described on page 8 under General Methods. The determination is quickly and easily made, using a few drops of the oil or melted fat. The standard temperature for reporting the refractive index is 25°C. in the case of oils and 40° for the solid fats, and it is desirable to make the readings at nearly these temperatures. If the temperature is slightly above or below the standard, the reading for edible oils should be corrected by 0.000365[1] for each degree difference, remembering that the index of refraction increases as the temperature decreases.

Although the Abbe refractometer is the one best adapted to general laboratory use, in the case of fats and oils somewhat more delicate readings can be obtained with the *butyro-refractometer*, an instrument specially designed for the edible fats and oils and restricted to the range of values which they cover. Directions for the use of this instrument and tables for converting its readings to indexes of refraction may be found in Leach-Winton's "Food Inspection and Analysis."

Notes.—The great value of the refractive index as an analytical constant lies in the ease with which the test may be applied and the small sample that is needed. For this reason it is often used in the routine examination of numbers of samples as a preliminary or sorting test. With oils in general, the refractive index varies as does the specific gravity, both increasing with an increased percentage of unsaturated acids and increasing molecular weights, a notable exception being butter fat, which, on account of the high proportion of acids of low molecular weight, has a lower refractive index than other animal fats, although its specific gravity is higher.

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[1] TOLMAN and MUNSON: *J. Am. Chem. Soc.*, **1902**, 754. According to Richmond, *Analyst*, **1907**, 44, the corrective value is nearer 0.00038. If either value be used, by making the determination at a temperature as near as practicable to the standard and yet having the fat liquid, the error in the final result will be inappreciable.

TABLE 20.—COMMON EDIBLE OILS IN THE ORDER OF THEIR REFRACTIVE INDEXES[1]

Soybean oil	1.475	Peanut oil	1.468
Poppyseed oil	1.473	Lard oil	1.467
Corn oil	1.472	Olive oil	1.467
Mustard oil	1.472	Lard	1.452
Sunflower oil	1.472	Palm oil	1.451
Rape oil	1.471	Tallow	1.451
Sesame oil	1.471	Cocoa butter	1.450
Cottonseed oil	1.471	Butter fat	1.447
Tea-seed oil	1.470	Coconut oil	1.441
Almond oil	1.469		

(at 60°C.)

[1] Average values at 25°C.; for the usual variations see Table 27, p. 199.

Melting Point. *a. Capillary-tube Method.*—Melt a small quantity of the fat at as low a temperature as possible and draw it up into several thin-walled capillary tubes about 3 cm. long. Place these on ice for not less than 12 hours. Attach one of the tubes thus prepared to a delicate thermometer graduated to tenths of a degree, using a small rubber ring clipped from a piece of tubing. The tube should be so attached that the fat is as close as possible to the bulb of the thermometer. The thermometer is supported by a cork or a clamp so that its bulb is immersed in water in a wide test tube, which in turn rests in the neck of a round-bottomed flask also containing water (Fig. 49). The water is heated gradually at a rate not exceeding 0.5° per minute until the fat melts. The temperature at which the fat becomes transparent is taken as the melting point. This should not be confused with the *softening point,* usually several degrees below the true melting point, at which the fat may gradually change its position in the capillary. The mean of several determinations should be taken as the final value.

b. Wiley's Method.[1]—On account of the difficulty in determining the precise point at which the fat becomes liquid, if exact results are desired, the following method is preferable. In the case of determinations on the fatty acids themselves, however, these being soluble in alcohol, the capillary-tube method must be employed.

1. Prepare disks of the fat by allowing several drops of the melted fat to fall from a height of about 15 to 20 cm. onto a smooth piece of ice floating in recently boiled distilled water or

[1] WILEY: "Agricultural Analysis," Vol. III, p. 324; *Assoc. Off. Agr. Chem.,* "Official Methods," **1935,** p. 407.

upon the surface of cold mercury. Thin disks about 1 cm. in diameter will be formed and can be removed by forcing the ice below the water, when the disks will float and can be taken up on a steel spatula or knife blade previously cooled in ice water.[1] The disks should preferably be allowed to stand in the refrigerator several hours before being used to determine the melting point,
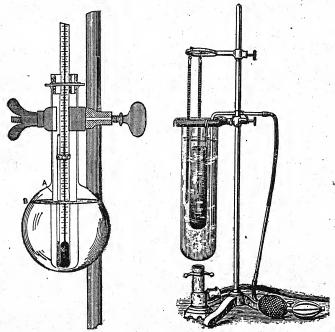
FIG. 49.—Melting-point appa-
ratus. (*Mulliken.*)

FIG. 50.—Wiley's melting-point
apparatus.

since the solid fat formed quickly by chilling will not show its true melting point.

2. Half fill a wide test tube with hot, recently boiled, distilled water, then carefully pour a nearly equal volume of hot, recently boiled alcohol down the side of the tube so as to form a layer above the water. Suspend the test tube in a wider tube or tall beaker nearly filled with water and provided with a bent glass

[1] A simple method of casting uniform disks of fat in a chilled perforated metal plate has been described by BAILEY: *J. Assoc. Off. Agr. Chem.*, **1924,** 88.

tube reaching nearly to the bottom through which air can be blown for stirring (Fig. 50). The test tube containing the alcohol and water is placed in the beaker containing water and a little ice and allowed to remain until cold. Drop in a disk of fat, which will sink to a point where the specific gravity of the alcohol-water mixture is the same as its own. Lower a delicate thermometer, reading to tenths of a degree, into the tube until the bulb is just above the disk and stir very gently with the thermometer. Slowly heat the beaker, keeping the water well stirred. When the disk begins to shrivel, indicating that the temperature is about 5° from the melting point, lower the thermometer until the fat particle is even with the center of the bulb and heat very slowly so that not less than 10 minutes are required for the temperature to rise the last 2°. Occasionally move the thermometer bulb gently around the disk of fat as the melting point is approached. The reading is taken when the fat becomes a sphere. Having determined the approximate melting point by a preliminary trial, duplicate determinations should agree within 0.2°.

It is important to use a very thin disk of fat and to add the alcohol to the water while both are still hot, since otherwise the mixture will contain many air-bubbles that will gather on the disk of fat as the temperature rises and finally force it to the surface.

Notes.—Since the fats are mixtures of various glycerides, they do not show the sharp melting-point characteristic of pure organic compounds, but first soften, then shrink in volume, and gradually form a transparent liquid. Hence different values may be recorded, depending on whether the beginning of fusion or the transparent point is observed, and to secure concordant results a uniform procedure must be followed.

TABLE 21.—COMMON EDIBLE FATS IN ORDER OF THEIR MELTING POINTS[1]

Tallow	45°C.
Lard	40°C.
Palm oil	40°C.
Cottonseed stearin	33°C.
Butter fat	32°C.
Cocoa butter	30°C.
Coconut oil	25°C.

[1] Average values, for usual variations see Table 27, p. 199.

If the fat consists mainly of glycerides of fatty acids of the saturated or acetic series, its melting point will increase as the

mean molecular weight of the mixed acids increases; whereas, if unsaturated acids are present, the melting point decreases proportionally.

CHEMICAL METHODS

Saponification Number.[1]—The saponification number, or the Koettstorfer number, as it is sometimes called from the originator of the process, is *the number of milligrams of potassium hydroxide required to saponify* 1 *gram of the oil or fat.*

Method.—Weigh about 5 grams of the oil or melted fat into a 200-cc. Erlenmeyer flask. This is best done by weighing 10 to 15 grams of the oil to the nearest centigram in a small beaker together with a small pipette or medicine dropper. The required amount of oil (1 gram is about 40 drops) is transferred by means of the dropper to the flask, taking care not to get any on the neck, the dropper is replaced and the whole reweighed. Add carefully from a pipette or burette 50 cc. of approximately 0.5N alcoholic potassium hydroxide,[2] close the flask with a cork carrying a straight glass tube several feet long, or place a small funnel in the neck of the flask, and boil gently for 30 minutes or until completely saponified. The solution at this point should be homogeneous, no oil separating when the boiling is interrupted. When saponification is complete, cool the flask, add 1 cc. of phenolphthalein solution, and titrate the excess of alkali with 0.5N hydrochloric acid.

Two blank determinations must be carried out at the same time, using similar flasks, 25-cc. portions of the alcoholic potassium hydroxide, and taking all precautions as to draining of pipette or burette, time and condition of boiling, etc., in order that a true correction may be made.

Since each cubic centimeter of 0.5N acid is equivalent to 28.05 mg. of KOH, the saponification number is found by sub-

[1] KOETTSTORFER: *Z. anal. Chem.*, **1879**, 199.

[2] Dissolve 40 grams of highest grade potassium hydroxide, preferably that which has been purified by crystallization from alcohol ("potash by alcohol") in 1 liter of alcohol which has been purified as follows: Reflux 1.2 liters of 95 per cent alcohol over 10 grams of potassium hydroxide and 6 grams of aluminum foil for about 30 minutes. Distill by heating on a steam bath, discarding the first 50 cc. of the distillate. Use an all-glass apparatus or keep all corks covered with tin foil (STOUT and SCHUETTE: *Ind Eng. Chem., Anal. Ed.*, **1933**, 100).

tracting the number of cubic centimeters of acid used in titrating the sample from the amount used for the blank, multiplying the result by 28.05 and dividing by the weight of oil taken.

Notes.—The fats and oils under consideration, being glyceryl esters of fatty acids, can be hydrolyzed into glycerol and the free fatty acids or decomposed by bases into glycerol and salts of the fatty acids.   A typical reaction of the latter kind, *saponification*, a *soap* being formed, would be

$$C_3H_5(C_{17}H_{35}COO)_3 + 3KOH \rightarrow C_3H_5(OH)_3 + 3C_{17}H_{35}COOK$$

    Stearin   Glycerol   Potassium stearate

The differences in saponification value found are of course due to the fact that esters of fatty acids of low equivalent weights will require more base for saponification than the same weight of those of higher equivalent weights.

The purest alcohol obtainable must be employed in the preparation of the alcoholic potash, since ordinary alcohol contains an appreciable amount of aldehyde, which in the presence of the potash forms the yellow aldehyde resin and gradually darkens, so that it is difficult to see the end point. · For this reason, the removal of the aldehyde gives a much more satisfactory solution. If the saponified oil is still too dark to titrate, better results may be secured by diluting it with 50 cc. of *neutral* alcohol or by using with the phenolphthalein an equal amount of a cold-saturated alcoholic solution of Alkali Blue 6B[1] (red with alkalies, blue with acids).

The saponification and titration should be carried out with as little access of air as possible, since the alkali solution readily absorbs carbon dioxide, a still further reason for making the blanks precisely parallel to the determination.

If desired, the titrated solution may be used for the determination of the per cent of insoluble fatty acids, pages 193 and 236.

The saponification number is, as would be expected, inversely proportional to the mean molecular weights of the fatty acids present, its principal value being, as a matter of fact, to indicate the presence of the lower fatty acids such as occur in coconut oil, butter, etc.   The following table shows the differences found in some of the common glyceryl esters of the edible oils:

[1] MARCUSSON: *Z. angew. Chem.*, **1911**, 1297.

TABLE 22.—SAPONIFICATION VALUES OF GLYCERYL ESTERS

	Saponification value	Molecular weight
Butyrin	557.3	302
Laurin	263.8	638
Palmitin	208.8	806
Stearin	189.1	890
Olein	190.4	884
Linolein	191.7	878
Linolenin	192.4	872
Arachidin	172.7	974

Since the natural oils contain more than one ester and these by no means in a pure state, the values actually obtained in practice do not show such differences as the above table might indicate. It must be borne in mind, further, that many of the glycerides present in fats and oils are *mixed* glycerides, *i.e.*, different acid radicals attached to the same glyceryl radical. The test is, however, of distinct value in showing the presence of glycerides of the "butter acids" or of unsaponifiable matter, such as mineral oils.

If from the saponification number be subtracted the *acid number* (see below) the difference will be the number of milligrams of alkali actually used in the saponification of the glyceryl esters, the so-called *ester number*.

The values of the common edible oils are found in the table below.

TABLE 23.—COMMON EDIBLE OILS IN ORDER OF THEIR SAPONIFICATION VALUES[1]

Coconut oil	253	Soybean oil	192
Butter fat	227	Almond oil	191
Palm oil	200	Sesame oil	191
Cocoa butter	197	Sunflower oil	191
Lard	197	Corn oil	191
Lard oil	195	Tea-seed oil	191
Beef tallow	195	Olive oil	190
Cottonseed stearin	194	Peanut oil	190
Poppyseed oil	193	Rape oil	174
Cottonseed oil	193	Mustard oil	173
Mutton tallow	193		

[1] Average values; for usual variations see Table 27, p. 199.

Free Fatty Acids. Acid Value.—Weigh about 20 grams of the oil or fat into an Erlenmeyer flask, add 50 cc. of 95 per cent alcohol that has been previously carefully neutralized to phenolphthalein with $0.1N$ sodium hydroxide, heat on the water bath nearly to boiling, and titrate with $0.1N$ alkali and phenolphthalein until the pink persists for at least 1 minute. It is necessary to shake thoroughly after each addition of alkali to secure complete extraction of the fatty acid from the immiscible oily layer.

If the solution is dark-colored, Alkali Blue 6B may be used in place of phenolphthalein, as suggested in the preceding method.

The result may be expressed as *percentage of oleic acid* (1 cc. $0.1N$ alkali = 0.0282 gram of oleic acid) or as the milligrams of potassium hydroxide required to neutralize the free fatty acids in 1 gram of oil (*acid number, acid value*).

Note.—The presence of free fatty acids is due ordinarily to decomposition of the glycerides caused by chemical treatment or bacterial action, accelerated by light and heat. The amount found in edible fats and oils is not very considerable, although in palm oil the quantity present may be as great as 75 per cent (calculated as palmitic acid). The determination is often used to distinguish between edible and non-edible olive oil, for fixing the customs duty.

Iodine Number.—The iodine number is *the number of grams of iodine absorbed by* 100 *grams of the oil.*[1] This constant is perhaps the most valuable of the general methods used for differentiating or identifying oils, in that it readily serves to indicate the group to which the oil belongs and is not so easily affected by slight changes in the oil as are some of the other constants.

The method depends upon the fact pointed out on page 172, that, unlike the fatty acids of the acetic series, the unsaturated acids of the oleic, linoleic or less saturated series, as well as their glyceryl esters, absorb halogens to form mainly addition products. Thus oleic acid, $C_{17}H_{33}COOH$, takes up 2 atoms of iodine and forms the *addition product,* diiodo stearic acid, $C_{17}H_{33}I_2COOH$; hence one molecular weight of the triglyceride olein would absorb 6 atomic weights of iodine, and one of linolenin would absorb 18 atomic weights of iodine.

[1] Although expressed as iodine, the substance actually absorbed is iodine chloride or iodine bromide, depending on the method employed.

Iodine itself is absorbed very slowly by the fat or oil; hence the reaction is ordinarily carried out in such a manner that the addition shall take place through the agency of iodine chloride or bromide. The method most widely used in the past was that of Hübl,[1] based upon the use of an alcoholic solution of iodine in the presence of mercuric chloride. A study of the mechanism of the Hübl process, however, led Wijs[2] to propose the use of iodine monochloride directly. This reagent, used in acetic acid solution, is more stable and acts more quickly than the Hübl reagent. Subsequently Hanus[3] suggested the use of iodine bromide instead of the chloride, as being easier to prepare and distinctly more stable than the Wijs reagent.

Both the Wijs and Hanus methods have advantages over the Hübl process and have practically taken its place. The Wijs method is more commonly used in England and Germany and is stated by Lewkowitsch to give results more nearly correct than the Hanus method. The latter, however, has been adopted as an official method by the Association of Official Agricultural Chemists and is considerably more convenient to use. In the case of the edible fats and oils, moreover, it gives entirely satisfactory results. In reporting results, however, it is best to state what method was used.

Hanus Method. *Reagents.—a. Iodine Solution:* Dissolve 13.61 grams of powdered iodine in 825 cc. of glacial acetic acid (99.5 per cent strength, showing no reduction with potassium bichromate). This is conveniently done by heating on the steam bath and adding the acetic acid in small portions, decanting each time after 10 minutes into a large bottle or flask. When cool decant the entire solution to be sure that no undissolved particles of iodine remain. Pipette 25 cc. into a flask, add 20 cc. of potassium iodide solution, *d*, 100 cc. of water, and titrate with the thiosulphate solution.

From a small graduate measure 3 cc. of bromine into another 200-cc. portion of the glacial acetic acid. Mix and pipette 5 cc., add potassium iodide and water as before, and titrate with the thiosulphate solution. Calculate the volume of bromine solution equivalent to the 800 cc. of iodine solution. Add this

[1] *Dingler's polytech. J.*, **1884**, 281.

[2] *Ber.*, **1898**, 750; *Z. Nahr.-Genussm.*, **1898**, 561.

[3] *Z. Nahr.-Genussm.*, **1901**, 913.

quantity to the iodine solution, mix well, and keep in a glass-stoppered bottle, preferably in the dark.   This final solution of iodine monobromide should have a slight excess only of either iodine or bromine.

b. Sodium Thiosulphate Solution (approximately $0.12N$): Dissolve 31 grams of the recrystallized salt in a liter of water.

c. Standard Potassium Bichromate Solution: Weigh out 3.8633 grams of pure potassium bichromate, dissolve in water, and dilute to 1 liter.   If the bichromate is pure and dry, 1 cc. of this solution should be equivalent to 0.01 gram of iodine.   The solution may be checked against a pure ferrous salt, containing a definite percentage of iron.

d. Potassium Iodide Solution: 150 grams of potassium iodide, free from iodate, per liter.

Standardizing the Thiosulphate Solution.—Pipette 20 cc. of the bichromate solution into an Erlenmeyer flask, add an equal volume of water, 10 cc. of potassium iodide solution, *d*, and 5 cc. of concentrated hydrochloric acid.   Titrate with the thiosulphate until the red color, due to the free iodine, has changed to a pale yellow, then add 2 cc. of a freshly prepared starch solution (1:200), and titrate cautiously until the blue color changes to a sea-green.

The reaction may be expressed:

$$K_2Cr_2O_7 + 14HCl + 6KI \rightarrow 2CrCl_3 + 8KCl + 7H_2O + 3I_2.$$

Process.—Weigh into a 300-cc. glass-stoppered bottle about 0.5 gram of a fat, 0.25 gram of salad oil or 0.10 to 0.20 gram of a drying oil, and dissolve in 10 cc. of chloroform.   Add 25 cc. of the iodine solution prepared as above from a pipette or glass-stoppered burette, taking care that none of the solution touches the neck of the bottle.   Carefully insert the stopper, rotate gently, avoiding spattering any of the solution on the stopper, and allow the bottle to stand for 30 minutes.   Make duplicate determinations, and in similar bottles carry out two blank determinations in exactly the same manner and measuring the same quantity of reagents.

At the end of 30 minutes carefully remove the stopper, add 20 cc. of potassium iodide solution, *d*, pouring it over the stopper, and 100 cc. of water.   Titrate immediately with the standardized sodium thiosulphate solution, which may be run in rapidly until

the solution becomes pale yellow. Then add 2 cc. of the starch solution and titrate to the disappearance of the blue color. Toward the end of the titration, stopper the bottle and shake vigorously in order to react with any iodine that may be dissolved in the chloroform. From the amount of thiosulphate solution employed, as compared with the blanks, calculate the percentage of iodine absorbed.

Example.—Weighed out 0.3978 gram of peanut oil; added 25 cc. of iodine solution. For titrating used 22.85 cc. of a thiosulphate solution of which 15.20 cc. were equivalent to 0.2 gram of iodine. In a blank determination 25 cc. of iodine required 48.60 cc. of thiosulphate.

From blank, 25 cc. iodine solution = 48.60 cc. thiosulphate.

$$\begin{array}{r} 48.60 \\ 22.85 \\ \hline \end{array}$$

0.3978 gram oil corresponds to $\overline{25.75}$ cc. thiosulphate.

25.75 cc. thiosulphate = $\dfrac{0.2 \times 25.75}{15.20}$ grams of iodine.

Since this amount of iodine is absorbed by 0.3978 gram of oil, 100 grams would absorb $\dfrac{25.75 \times 0.2 \times 100}{0.3978 \times 15.20} = 85.1$ grams.

The iodine number, then, is 85.1.

Notes.—Care should be taken that the reagents are pure, especially the acetic acid, which should show no green color when warmed on the water bath with potassium bichromate and sulphuric acid.

It is essential that a considerable excess of iodine, at least twice the amount absorbed, should be present. For this reason, when using a definite amount of the iodine solution, it is necessary to regulate accordingly the amount of oil or fat weighed.

It is best to measure out the various quantities of the iodine solution required for duplicate determinations and for the blanks within a short interval of time, since, on account of the very high coefficient of expansion of acetic acid, the apparent strength of the solution is materially altered by slight changes in temperature.

When the titrated solution is allowed to stand it frequently becomes blue again, owing probably to the splitting off of iodine from the compound formed, the reaction being to some extent a reversible one. The first end point should be the one taken.

The active substance involved in the process originally described by Hübl is undoubtedly iodine monochloride, which is formed when the iodine and mercuric chloride solutions are mixed, as expressed by the equation:

$$HgCl_2 + 2I_2 \rightleftharpoons HgI_2 + 2ICl.$$

It is probably the iodine monochloride that is absorbed by the fat, this being less stable than the iodine molecule, although from an analytical standpoint it is immaterial whether this or free iodine is the active agent, since upon adding potassium iodide an equivalent amount of iodine is liberated and titrated.

$$ICl + KI \rightarrow KCl + I_2.$$

Thus, in the blank determinations, the amount of free iodine titrated must be the amount present as free iodine in the original solution; since none has been removed. A logical development of the conception of iodine monochloride as the active agent was the proposal of Wijs to use this directly, or the use of iodine bromide as suggested by Hanus.

Wijs Method. *Reagents.—a. Iodine Solution:* Dissolve 13 grams of resublimed iodine in 1 liter of glacial acetic acid (99.5 per cent strength). Pass in washed and dried chlorine gas until the original thiosulphate titration of the solution is not quite doubled. The most convenient way to do this is to dissolve the iodine in the acetic acid by warming, as described on page 185, and set aside a small portion of this solution while chlorine is passed into the remainder until the halogen content is doubled. Ordinarily it will be found that by passing the chlorine into the main part of the solution until the characteristic color of free iodine has just been discharged, there will be a slight excess of chlorine which is corrected by the addition of the necessary amount of the unchlorinated portion until all the free chlorine has been removed. A slight excess of iodine does little or no harm, but excess of chlorine must be avoided.

Preserve the solution in glass-stoppered amber bottles, sealed with paraffin until ready for use. Mark the date on which the solution was prepared on the bottle and do not use Wijs solution that is more than 30 days old. The reagent is much more sensitive to light than is the Hanus solution.

b. Potassium iodide, potassium bichromate, and sodium thiosulphate solutions as in the Hanus method, page 186.

Process.—Proceed as in the Hanus method, observing the same precautions as regards time of reaction, loss of iodine, and carrying out blank determinations as there described. During the absorption by the oil, the bottles are best kept in the dark.

Notes.—Absorption of iodine from the Wijs solution appears to take place with greater promptness and be complete in a shorter time than from the Hanus. Results by the Wijs method are also in better agreement in the case of oils showing a high iodine absorption, and the results run somewhat higher for the same length of time. The difference is especially marked with some of the drying oils, especially tung oil. For this reason the Wijs method has been adopted as standard by the Committee on Analysis of Commercial Fats and Oils of the Division of Industrial Chemists and Chemical Engineers of the American Chemical Society.[1] With fats and oils having iodine numbers less than 100, the differences in the results obtained by the two methods are no greater than the variations that may be found in the oil itself. A comparison of the values given by the three methods on the common edible oils has been made by Tolman and Munson[2] and by Hunt.[3]

TABLE 24.—COMMON EDIBLE OILS IN ORDER OF THEIR IODINE NUMBERS[1]

Poppyseed oil	136	Peanut oil	93
Soybean oil	128	Tea-seed oil	89
Sunflower oil	127	Olive oil	85
Corn oil	120	Lard oil	75
Cottonseed oil	110	Lard	58
Sesame oil	108	Palm oil	55
Mustard oil	104	Tallow	40
Rape oil	101	Cocoa butter	35
Almond oil	97	Butter fat	32
Cottonseed stearin	96	Coconut oil	9

[1] Average values: for the usual variations see Table 27, p. 199.

Thiocyanogen Value.—A figure somewhat similar to the iodine value can be obtained by allowing the unsaturated acids to absorb thiocyanogen $(CNS)_2$ in a manner analogous to the

[1] *Ind. Eng. Chem.*, **1919**, 1161.
[2] *J. Am. Chem. Soc.*, **1903**, 244.
[3] *J. Soc. Chem. Ind.*, **1902**, 454.

absorption of iodine. Linoleic acid or its glyceride combines with thiocyanogen only at one of the two double bonds. By means of this newer value, suggested originally by Kaufmann[1] and studied considerably since, taken in connection with the Wijs iodine number, it is possible to determine fairly definitely the percentages of oleic, linoleic, linolenic, and saturated acids in an oil, provided other unsaturated acids are absent. The method is difficult and not well suited for a routine test but affords information sometimes essential. Details may be found in Jamieson's "Vegetable Fats and Oils" or Jacobs' "Chemical Analysis of Foods and Food Products."

Acetyl Value.—This, as determined on oils, is primarily a test for the amount of hydroxylated fatty acid, the hydrogen of the hydroxyl group being replaced by acetyl through heating with acetic anhydride. The most important of these acids found in oils is *ricinoleic acid*, $C_{18}H_{34}O_3$, the glyceride of which makes up the greater part of castor oil. The test is really one for castor oil or for certain lubricating oils and has but little value in the examination of edible oils; hence, although it was described in previous editions of this book, it is now omitted. A good discussion of the test will be found in Mahin's "Quantitative Analysis," 4th ed.

Maumené Number.—The Maumené number[2] is *the number of degrees Centigrade which the temperature rises when 10 cc. of strong sulphuric acid is mixed with 50 grams of oil.* The exact strength of the acid employed is naturally of great importance, and to secure more uniform results it has been proposed[3] to compare the rise in temperature with that produced by water under the same conditions, the latter being taken as 100. The number stated in this way is called the *specific Maumené number* or "specific temperature reaction." Thus, if 50 grams of cottonseed oil mixed with 10 cc. of sulphuric acid showed an increase in temperature from 22°C. to 97°C., its Maumené number would be 75; if 50 grams of water under the same conditions showed a rise of 46°, the specific Maumené number would be $^{75}/_{46} \times 100 = 163$.

Procedure.—Use a rather tall beaker of about 150-cc. capacity and insulate it by placing it in a larger beaker or agateware cup

[1] *Z. Untersuch. Lebensm.*, **1926**, 15.

[2] MAUMENÉ: *Compt. rend.*, **1852**, 572.

[3] THOMSON and BALLANTYNE: *J. Soc. Chem. Ind.*, **1891**, 234.

and packing the space between with cotton waste or felt. The insulation should be sufficient to prevent the outer vessel becoming perceptibly warm during a test. The oil to be tested, the water for the control test, and the sulphuric acid should all be at the same temperature, within a few degrees of 20°C. This may be brought about by immersing them in a tank of water at the desired temperature or more simply in most cases by letting them stand side by side on the laboratory desk for several hours.

Place 50 cc. of water in the beaker, immerse the thermometer, and read the temperature to the nearest 0.1°. Add 10 cc. of the acid, running it slowly from a pipette, and stir the mixture thoroughly with the thermometer. At intervals of a few seconds hold the thermometer in the center of the beaker and read the temperature. Record the highest point at which the thermometer remains constant for any appreciable time. As soon as this has been done throw out the mixture of water and acid in order not to heat the apparatus unnecessarily. When the initial temperature has been restored again repeat the determination. Duplicate determinations on water should not differ by more than 0.5°.

Having determined the rise of temperature with water, dry the beaker, weigh into it 50 grams of oil within a drop or so, and carry out the test in exactly the same way as before, special care being taken to stir the mixture thoroughly if it becomes thick and gummy. Empty the beaker while still warm and wipe it thoroughly with cotton waste.

Notes.—The results obtained by this method are constant for a particular oil only when the details of manipulation are always rigidly observed, slight differences in the manner of carrying out the test causing serious discrepancies in the result obtained. For example, a difference of 5° has been noted when in one case the mixture was constantly stirred, and in another the oil was stirred until all the acid has been added and then the thermometer was held stationary in the center of the beaker. Much study has been given to the details of the method, especially with regard to the strength of the acid and the effect of diluting the mixture with some inert material. Since, however, the results reported by various authorities may differ quite considerably, having been obtained by slightly different methods, it is always best to compare a sample with one of known purity under exactly similar

conditions.[1]   In testing edible oils the strongest sulphuric acid
obtainable should be used; for fish and drying oils where the
action is more violent, a more dilute acid may be employed or the
oil may be diluted, using either a mineral oil or carbon tetra-
chloride, preferably the latter.

The results given by the Maumené test for different oils
follow in general the iodine numbers, both being mainly depend-
ent on the proportion of glycerides of the unsaturated fatty acids.
An important difference, and one in which lies the greatest value
of the Maumené number, is the fact that oils that have been
oxidized by standing show an increased Maumené number, but a
decreased iodine value.   This is illustrated by the following
figures:[2]

Oil	Iodine number	Specific Maumené number
Olive oil......................................	83.8	100
Olive oil after exposure.....................	77.3	127
Lard oil......................................	73.3	106
Lard oil after exposure.....................	56.2	141
Cottonseed oil	105.2	171
Cottonseed oil after exposure	90.2	217

The Maumené number might be especially helpful in cases like
these in showing that the abnormal values obtained for certain of

TABLE 25.—COMMON EDIBLE OILS IN ORDER OF THEIR SPECIFIC MAUMENÉ
NUMBERS[1]

Poppyseed oil..............	220	Rape oil.....................	140
Corn oil....................	180	Peanut oil...................	125
Sunflower oil................	167	Almond oil...................	110
Mustard oil.................	160	Olive oil....................	100
Cottonseed oil..............	155	Lard oil.....................	90
Sesame oil..................	155	Soybean oil..................	90

[1] Average values; for the usual variations see Table 27, p. 199.

the "constants" were due not to adulterants but to a change in
the oil itself.   It is not a routine test but is included here because
of its special value in detecting certain adulterants of olive oil.

[1] See among others the following papers: SHERMAN, DANZIGER, and KOHN-
STAMM: *J. Am. Chem. Soc.*, **1902**, 266; RICHTER: *Z. angew. Chem.*, **1907**,
1613; TORTELLI: *Chem.-Ztg.*, **1909**, 134, 171, 184; WILISCH: Inaug. Diss.,
Augsburg, **1912**.

[2] SHERMAN: "Organic Analysis," 2d ed., p. 211.

Reichert-Meissl Number.—(Volatile fatty acids.)

Hehner Number.—(Insoluble fatty acids.)

These two methods, although possibly of general application, are of greatest value in testing the purity of butter fat, and will therefore be described under the special methods for the analysis of butter fat on pages 230 to 236.

Examination of the Mixed Fatty Acids.—In cases where the examination of the oil or fat has not yielded sufficiently definite information, and a sufficient quantity of the sample is available, it may be of advantage to determine certain of the constants of the fatty acids themselves. These correspond in a general way to the values determined on the individual oils, but in some cases, as with the melting and solidifying points, are more characteristic for the acids than for their glycerides.

Preparation of the Free Acids.—Saponify 25 grams of the oil by boiling with 20 cc. of potassium hydroxide solution (10 grams KOH in 20 cc. of water) and 20 cc. of alcohol. Use an Erlenmeyer flask provided with a cork carrying a straight glass tube to serve as a condenser and heat on the steam bath. Evaporate the alcohol and dissolve the pasty residue in 200 or 300 cc. of hot water. Add an excess of hydrochloric or sulphuric acid and boil gently until the fatty acids form a clear oily layer at the top of the liquid. Siphon off the aqueous layer or separate by a separatory funnel and wash several times with hot distilled water until free from mineral acid. Separate as thoroughly as possible from the water and pour the melted fatty acids through a plaited filter in a funnel which is kept warm. On the fatty acids thus obtained, the iodine number, melting point, or refractive index may be determined by the methods previously described.

Note.—To test the completeness of the saponification 3 cc. of the fatty acids are dissolved in 15 cc. of strong alcohol and 15 cc. of ammonia added. If an appreciable amount of fat has escaped saponification the mixture will become turbid. If the original fat contained much unsaponifiable matter, turbidity might be due to that cause also. This will not usually occur, however, with edible fats and oils.

Solidifying Point of Fatty Acids.—"*Titer Test.*"—The determination of the solidifying point of the mixed fatty acids is much more characteristic than the melting point and is largely used for the commercial testing and valuation of fats. The method of

the Association of Official Agricultural Chemists,[1] which is a modification of the original procedure proposed by Dalican, gives results that are 0.2 to 0.3° higher than those obtained by the earlier process but are probably more accurate on account of the more thorough drying of the fatty acids.

a. Apparatus. Standard Thermometer.—In order to secure uniform results, the specifications for the thermometer are rigidly stated. It is unnecessary, however, to detail them here because suitable thermometers can be purchased. It might be mentioned, however, that new and somewhat more desirable specifications have been drawn up.[2]

b. Determination.—Saponify 75 grams of fat in a metal dish with 60 cc. of 30 per cent sodium hydroxide (36° Baumé) and 75 cc. of 95 per cent by volume alcohol or 120 cc. of water. Boil to dryness, with constant stirring to prevent scorching, over a very low flame or over an iron or asbestos plate. Dissolve the dry soap in 1 liter of boiling water, and if alcohol has been used boil for 40 minutes in order to remove it, adding sufficient water to replace that lost in boiling. Add 100 cc. of 30 per cent sulphuric acid (25° Baumé) to free the fatty acids, and boil until they form a clear, transparent layer. Wash with boiling water until free from sulphuric acid, collect in a small beaker, and place on the steam bath until the water has settled and the fatty acids are clear; then decant them into a dry beaker; filter, using a hot-water funnel, and dry 20 minutes at 100°C. When dried, cool the fatty acids to 15 or 20°C. above the expected titer and transfer to the titer tube, which is 25 mm. in diameter and 100 mm. in length (1 by 4 in.) and made of glass about 1 mm. in thickness. Place in a 16-oz. widemouthed bottle of clear glass, about 70 mm. in diameter and 150 mm. high (2.8 by 6 in.), fitted with a cork, which is perforated so as to hold the tube rigidly when in position. Suspend the thermometer, graduated to 0.10°C., so that it can be used as a stirrer, and stir the mass slowly until the mercury remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the mass, and observe the rise of the mercury. The highest point to which it rises is recorded as the titer of the fatty acids.

Test the fatty acids for complete saponification as on page 193.

[1] "Official Methods of Analysis," **1935**, p. 408.
[2] *J. Assoc. Off. Agr. Chem.*, **1936**, 95, 418.

Note.—It is essential that the details of the process be strictly followed and especially that the fatty acids be dry. Duplicate determinations should agree easily within 0.1°; variations in the results obtained by different chemists are due mainly to differences in the method of stirring during the test.

A more rapid but somewhat more expensive process for preparing the fatty acids is as follows: Heat 75 cc. of glycerol-potash solution (250 grams of potassium hydroxide in 1 liter of high-test glycerol) to 150°C. and add 50 cc. of the melted fat. Stir the mixture well and continue heating for 10 or 15 minutes, or until the melt is homogeneous, at no time allowing the temperature

TABLE 26.—COMMON EDIBLE OILS IN ORDER OF TITER TESTS OF MIXED FATTY ACIDS[1]

Oil or fat	°C.	Oil or fat	°C.
Cocoa butter	49.0	Coconut oil	23.0
Mutton tallow	45.0	Sesame oil	22.5
Beef tallow	44.0	Olive oil	20.0
Palm oil	43.0	Corn oil	19.0
Cottonseed stearin	38.0	Sunflower oil	17.5
Butter fat	35.5	Soybean oil	16.0
Lard	35.5	Poppyseed oil	15.8
Cottonseed oil	33.0	Rape oil	13.0
Peanut oil	28.5	Almond oil	11.6

[1] Average values; for the usual variations see Table 27, p. 199.

to exceed 150°C. Allow to cool somewhat and carefully add 50 cc. of 30 per cent sulphuric acid. Add hot water and heat until the fatty acids separate out perfectly clear. Draw off the acid water and wash the fatty acids with hot water until free from mineral acid; then filter and heat to 130°C. as rapidly as possible while stirring. Transfer the fatty acids, when somewhat cooled, to a titer tube and complete the test as described above.

Unsaponifiable Matter.—The amount of unsaponifiable matter, by which is meant all substances that may occur in fats that are insoluble or are incapable of forming soluble soaps with alkalies, is usually not over 1 to 2 per cent in edible fats and oils if pure. The presence of any appreciable quantity will be indicated if the solution of the soap in alcoholic potash be diluted with warm water by the appearance of oily drops or of a whitish cloud.

Determination.[1]—Weigh 3 grams (to nearest centigram) of the fat or oil into a 200-cc. flask and boil for 1 hour under a reflux condenser with 25 cc. of alcoholic potash (see page 181). Transfer to a stoppered 100-cc. graduated glass cylinder and make up to 50 cc. with cold water. Add 30 cc. of redistilled petroleum ether (boiling point below 75°C.) and shake vigorously. Draw off the petroleum ether layer by a siphon, or as described on page 131. Repeat the operation with six more portions of petroleum ether. Place the 210 cc. of petroleum ether in a separatory funnel and wash it three times with 20-cc. portions of 10 per cent alcohol by volume, shaking vigorously each time. Transfer the petroleum ether to a tared flask, evaporate (*away from a flame*), and dry the residue in the water oven to constant weight.

Notes.—The relatively simple method described here will be satisfactory for edible oils. In case of controversy, the more detailed directions of the Fat Analysis Committee of the American Chemical Society may be followed.[2]

If the oil or fat has been extracted by ether or petroleum ether from a food product, the unsaponifiable matter may be considerable in amount and be of quite diverse character, including resinous substances, paraffin, or mineral oils. If the oil or fat, on the other hand, is examined as such and is reasonably pure, the unsaponifiable matter is ordinarily quite insignificant in amount, consisting almost entirely of *cholesterol* or *phytosterol*. In the case of olive oil a characteristic unsaturated hydrocarbon, *squalene*, $C_{30}H_{50}$, has been found to occur in the unsaponifiable matter in much greater quantity than in other edible oils.[3] Typical results in per cent of crude squalene are: Olive oil 0.41 to 0.54, peanut oil 0.07, rape oil 0.05, apricot kernel oil 0.02. For details of the method suggested, which has possibilities, the second paper (abstracted in *Analyst*, **1939**, 354) should be consulted.

Cholesterol and phytosterol are isomeric monohydric alcohols of the general formula $C_{27}H_{45}OH$, the former being characteristic of the animal fats, as the latter is of the vegetable oils. The

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[1] Spitz and Honig: *J. Soc. Chem. Ind.*, **1891**, 1039.

[2] *Ind. Eng. Chem.*, **1919**, 161, 1101; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 420.

[3] Thorbjarnarson and Drummond: *Analyst*, **1935**, 23; Grossfeld and Timm: *Z. Unters. Lebensm.*, **1939**, 249.

fact that they occur in the different classes of oils becomes at times of analytical importance when it is required to decide whether a given sample is of animal or vegetable origin, or whether a mixture of animal or vegetable fats or oils is present, as coconut oil in butter.

This can be done by noting the character of the crystals obtained by slow evaporation of the purified materials from alcohol,[1] or better in the case of mixtures by a determination of the melting point of the acetyl esters, the so-called "phytosteryl acetate test."[2]

Probably the most characteristic of the plant sterols, known under the general classification of *phytosterol* is *sitosterol*, isomeric with the formerly reported phytosterol and probably identical with it.

The term "phytosterol" is now generally applied to a group of isomeric vegetable sterols of which *sitosterol* is the most prominent. Cholesteryl acetate melts at 114°C.; "phytosteryl" acetate at 125 to 137°C., which obviously points to the fact that the "phytosterol" obtained from vegetable oils is a mixture. Anderson and his coworkers[3] have prepared various isomeric sterols and a highly purified sitosteryl acetate melted at 130 to 131°C.

The sterols may be separated from the oil or fat by precipitation with the glucoside *digitonin*.[4]

$$C_{27}H_{45}OH + C_{55}H_{94}O_{28} \rightarrow C_{82}H_{140}O_{29}$$

Cholesterol   Digitonin   Digitonin cholesteride

The sterols are converted to the corresponding acetates by heating with acetic anhydride and the melting point of the purified and recrystallized acetate determined. The method is rather delicate and requires some experience before definite conclusions can be drawn.[5]

[1] Börmer: *Z. Nahr.-Genussm.*, **1898**, 31, 544; Lewkowitsch: *J. Soc. Chem. Ind.*, **1899**, 557; Zetsche: *Pharm. Zentralhalle*, **1898**, No. 49.

[2] Börmer: *Z. Nahr.-Genussm.*, **1901**, 865, 1070; **1902**, 1018; Gill and Tufts: *J. Am. Chem. Soc.*, **1903**, 251, 254, 498.

[3] *J. Am. Chem. Soc.*, **1924**, 1717; **1926**, 2976.

[4] Windaus: *Chem.-Ztg.*, **1913**, 1001.

[5] Detailed directions for the rather complicated procedure will be found in *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 418.

Special Tests.—Besides the general methods described above there are numerous tests that are specific for certain oils, depending upon the color reactions of impurities or upon the presence of characteristic constituents. Some of the more important of these will be described under the typical examples that are discussed, olive oil and butter fat.

OLIVE OIL

Olive oil has been selected as typical of edible oils. This is used in enormous quantities as a salad and table oil, especially in the case of the best flavored and highest grades. Large quantities of edible oil of a somewhat lower quality are used for packing sardines and similar fish. Inferior grades are used as burning oil, for lubricating, and in the textile industries and soap making.

Source.—Olive oil is prepared by expression or extraction from the fruit of the olive tree, *Olea europea sativa.* The best grades are obtained from Italy, Spain, and other countries bordering on the Mediterranean, although considerable quantities are produced in southern California and Africa.

The olives are gathered just before they are ripe, since the oil at that time is of the highest quality. The yield of oil is ordinarily from 40 to 60 per cent, although the fruit grown in California seldom yields over 25 per cent. The quality of oil obtained is variable, depending on the care used in picking and storing the fruit. The oil with the finest flavor, and hence most highly valued as an edible oil, is that obtained from ripe, hand-picked olives by using moderate pressure and is known as "virgin" or "sublime" oil. Lower grades, but still largely used as edible or salad oils, are obtained by a second pressing, after the residue has been ground and mixed with water. The oils obtained by further pressing the residue or by extraction with carbon bisulphide are not used as edible oils but for technical purposes.

Properties.—The value of olive oil, as is true of the edible oils in general, is largely dependent on its color, freedom from sediment, and, above all, on the taste. The color of genuine oil may vary from very pale yellow to golden; the oils of lower grades, owing to the more extended treatment of the pulp, contain enough dissolved chlorophyll to impart a distinct greenish tinge. Cases have been reported in which the green color was due to the addi-

TABLE 27.—CONSTANTS OF EDIBLE FATS AND OILS

Name	Specific gravity 15.5°C / 15.5°C	Refractive index (15.5°C)	Melting point, °C	Saponification number	Iodine number	Maumené number	Spec. temp. number	Reichert-Meissl number	Hehner number	Fatty acids		
										Melting point, °C	Titer °C	Iodine number
Almond oil	0.914–0.920	1.472–1.475		188–195	93–100	51–54	95–120	0.5	96.0	13.0–14.0	10.0–11.5	93.5–96.5
Butter fat	0.930–0.940	1.445–1.449[1]	28–35	221–233	20–38			25.0–33.0	86.5–89.5	38.0–42.0	34.0–37.0	28.0–30.0
Castor oil	0.960–0.967	1.479–1.481		175–185	82–90	46–47	84–94	1.0–2.0		13.0	3.0	87.0–88.0
Cocoa butter	0.950–0.975	1.449–1.451[1]	28–33	192–202	32–38			0.2–0.8	94.5	48.0–52.0	48.0–49.0	33.0–38.0
Coconut oil	0.926–0.926	1.439–1.443[1]	22–27	246–260	8–10			6.6–8.4	82.5–90.5	24.0–27.0	21.0–25.0	8.4–9.0
Cod-liver oil	0.922–0.930	1.479–1.485		180–190	135–175	113–116	240–280	0.3–0.6	95.5	22.0–25.0	14.0–19.0	164.0–171.0
Corn (maize) oil	0.921–0.927	1.475–1.477		187–193	115–124	79–83	160–200	0.3–3.5	93.0	18.0–21.0	19.0	113.0–115.0
Cottonseed oil	0.920–0.925	1.473–1.476		191–196	105–115	75–80	140–175	0.7–0.9	95.0–96.0	35.0–40.0	31.0–35.0	110.0–115.0
Cottonseed stearin	0.918–0.923		26–40	194–195	89–103				96.0	27.0–30.0	35.0–42.0	94.0–96.0
Lard	0.934–0.938	1.450–1.454[1]	36–45	195–200	46–66	48		0.2–0.6	96.0	35.0–46.0	33.0–38.0	63.0–66.0
Lard oil	0.915–0.916	1.469–1.472		193–198	50–65	25–35	80–100		95.0–95.0			95.0–109.0
Menhaden oil	0.925–0.931	1.479–1.482		188–193	67–82	47	305–360	1.0	97.0	15.0–17.0		109.0–120
Mustard oil	0.915–0.919	1.474–1.477		171–176	148–172	123–128	140–180			20.0–27.0		86.0–90.0
Olive oil	0.915–0.918	1.471–1.472		185–196	94–113	42–44	90–110	0.6	95.0–96.0		17.0–23.0	52.0–58.00
Palm oil	0.921–0.924	1.450–1.452[1]	27–43	196–204	53–57	42–46		0.8–1.9	94.5–97.0	47.7–50.0	40.0–45.0	96.0–103.0
Peanut oil	0.917–0.920	1.471–1.474		186–194	85–100	49–56	105–140	0.5	95.0–96.0		28.0–29.0	139.0
Poppyseed oil	0.924–0.926	1.476–1.478		190–196	133–139	86–88	200–240	0.0	95.0–96.0		15.4–16.2	
Rape (colza) oil	0.913–0.917	1.474–1.476		170–179	97–105	50–60	130–150	0.0–0.6	94.5–96.0		12.0–14.0	97.0–105.0
Sesame oil	0.921–0.925	1.474–1.476		188–193	103–112	63–70	140–175	1.2	95.0–96.0		21.0–24.0	109.0–112.0
Soybean oil	0.922–0.928	1.475–1.478		188–194	130–138	87–88		0.5–0.8				
Sunflower oil	0.924–0.926	1.474–1.478		188–194	120–135	68–75	167		95.5		13–15	124.0–134.0
Tallow (beef)	0.860[2]	1.449–1.452[1]	43–48	193–198	35–45			0.2–0.5	95.0–96.0	43.0–47.0	17.0–18.0	40.0–43.0
Tallow (mutton)	0.858–0.860[2]	1.451[1]	44–47	192–195	32–45			0.3	95.0–96.0	47.0–49.0	43.0–44.0	34.0–36.0
Tea-seed oil	0.915–0.919	1.470–1.473		190–195	80–87			0.3–1.0	95.0–96.0	23–33	43.0–46.0	

[1] At 60°C.   [2] At 15.5°.

tion of copper salts. This may be detected by dissolving the oil in ether and shaking with dilute sulphuric acid. Copper, if present, will be found in the acid aqueous layer and can be detected or determined quantitatively by the usual methods. Slight variations in flavor are not of importance in the chemical examination, although they may be the prime factors in the valuation of a sample.

Chemically, the principal constituents are the glycerides of oleic and palmitic acids together with a smaller proportion of linoleic acid. The amount of free fatty acids should be less than 0.5 per cent in an edible oil, although the technical oils may contain up to 25 per cent, depending on how long the oil is left in contact with the pulp during manufacture. The unsaponifiable matter, which varies in amount from 0.5 to 1.0 per cent, is largely phytosterol. (See also page 196.)

When the oil is cooled to about 2°C. it deposits palmitin as a white, granular solid, and if kept in an ice box for a week or so will in many cases become quite solid. Tolman and Munson[1] have pointed out that California olive oil shows much less tendency to become solid than do the French and Italian oils.

Forms of Adulteration.—On account of its relatively high price and because it is in great demand, olive oil is very liable to adulteration with other oils. Even the lower grades of edible oil, which are not so readily salable on account of inferior flavor, may be, for that very reason, mixed with a cheaper, bland oil in order to disguise the unpleasant taste. The blending of lower grades or cheaper olive oil to extend the supply of a recognized high-grade oil is adulteration in the strict sense of the term, but is unfortunately practically impossible of detection by analysis. More "Lucca" oil has been exported under that name than could be produced in the whole province of Tuscany, but the evidence in such cases is statistical rather than chemical and obviously inapplicable to individual shipments or brands. It has been not uncommon to find containers which originally contained genuine olive oil used again with 90 per cent cottonseed oil to take advantage of the original labeling and general appearance.

The most common adulterants are cottonseed, peanut, corn, and lard oils. Tea-seed oil has been a common adulterant within a year or so. Less frequently employed are sesame, rape,

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **77**, p. 49.

poppy-seed, sunflower, mustard, and even coconut and fish oils. Adulteration has in a sense kept pace with analytical methods, and mixtures are now more commonly used as adulterants than single oils as before. The oil may be sold as "salad oil," not constituting an adulteration unless the label or statements of the dealer convey the impression of olive oil. Sold in this way quite commonly of late have been rape (colza), soybean, sunflower, and mustard oils.

Analytical Methods.—For the detection of these added oils both quantitative tests and when available qualitative reactions should be employed. Of the general quantitative methods that have already been described (pages 174 to 197), the most useful are the specific gravity or refractive index, the iodine number and saponification value. In the majority of cases it will be advisable to determine carefully at least three of these constants in addition to the specific qualitative tests that are described below under the individual adulterants. It is of course possible to use a mixture of oils so skillfully that the ordinary limits for olive oil shall not be exceeded, hence the actual clue to the adulteration may be furnished by the qualitative tests alone, but the evidence should in all cases be confirmed by a careful study of the quantitative results, both directly and in their relation to one another.

In doubtful cases an examination of the fatty acids, especially as regards their melting point and iodine number, may be advisable.

A reasonably safe procedure to follow for the systematic examination of olive or "salad" oils would be to determine first the refractive index, then the iodine value and saponification number of the sample. A comparison of the values obtained in these three tests with those given in the tables on pages 199 and 203 should give a good idea whether the oil is presumably genuine or adulterated, and point in some cases to the probable adulterant. These tests should then be followed by the specific reactions for cottonseed, sesame, peanut, and tea-seed oils as given below. It cannot be too strongly emphasized that, with all the color reactions and special tests, simultaneous tests should be made also upon genuine olive oil and upon a mixture of olive and a reasonable proportion of the oil in question. This is especially necessary if the reactions are being tried for the first time. If

the quantitative results still appear abnormal and no adulterant has been shown, one of the less commonly used oils as lard, rape, sunflower, soybean, or fish oil may be present, or the possibility of the addition of heated cottonseed oil should not be overlooked. Study carefully the characteristics of genuine olive oil, as given on this page, and consult the authorities listed at the end of the chapter, especially Jamieson,[1] Allen[2] and Lewkowitsch-Warburton.[3]

INTERPRETATION OF RESULTS

Characteristics of Genuine Olive Oil.—Olive oil is a typical non-drying oil, hence is characterized by low iodine and Maumené numbers, these being lower than would be found normally in any oil that would be used as an adulterant. Restricting the range of variation of the iodine number to that occurring in *edible* oils, this determination alone should show by a distinctly high value the presence of 5 per cent or more of a drying oil like poppy, or 15 per cent of cottonseed, rape, corn, or sesame oil. Peanut and tea-seed oils, being more nearly like olive oil in iodine number, are not readily detected in this way.

The range of values given in Table 27, page 199, covers practically all that would be met in an edible olive oil, although higher or lower values would undoubtedly be found in commercial oils. An excellent idea of the extreme range found in edible olive oil of the kinds most largely sold in this country may be gained from Table 28, compiled from the results of Tolman and Munson.[4] Other analyses, especially of Tunisian oils, will be found in the authorities listed on page 253.

Possibly the most noticeable feature of these analyses is the rather wide range that certain of the so-called "constants," as the iodine number, Maumené number, and melting point of the fatty acids, exhibit in oils free from adulteration. This strengthens still further the statement already made that the quantitative results obtained in the examination of an oil should be considered with relation to one another as well as individually. For instance, an oil with an iodine number approaching the maximum given in the table for California oils might be a

[1] "Vegetable Oils and Fats."
[2] "Commercial Organic Analysis," 5th ed., Vol. II.
[3] "Oils, Fats and Waxes."
[4] U. S. Dept. Agr., *Bur. Chem. Bull.* **77.**

TABLE 28.—ANALYSES OF OLIVE OIL

Source		Specific gravity	Ref. index 15.5° C.	Maumené number*	Specific temperature reaction	Iodine number	Saponification number	M. pt. of fatty acids	Free fatty acid as oleic, per cent.
California oils of known purity	Max.	0.9180	1.4718	52.1	109.7	89.8	194.4	31.0	8.21†
(383 samples).	Min.	0.9162	1.4703	38.0	94.5	78.5	189.3	19.2	0.20
	Avg.	0.9170	1.4713	46.9	101.8	85.3	190.9	22.9	1.20
Italian oils of known purity	Max.	0.9180	1.4713	49.1	104.7	86.1	192.0	29.3	2.79
(18 samples).	Min.	0.9155	1.4705	39.6	95.6	79.2	189.6	21.6	0.57
	Avg.	0.9163	1.4709	44.9	99.1	81.6	190.9	25.5	1.11
California commercial oils, not	Max.	0.9177	1.4717	51.0	......	86.5	194.9	26.2	3.96
adulterated (12 samples).	Min.	0.9152	1.4705	41.0	......	79.2	190.5	24.5	0.29
	Avg.	0.9165	1.4710	45.5	......	82.2	192.7	20.7	1.95
Italian commercial oils, not	Max.	0.9179	1.4712	48.8	108.4	84.5	196.6	30.4	5.30
adulterated (56 samples).	Min.	0.9151	1.4701	39.8	88.4	77.5	190.1	21.0	0.70
	Avg.	0.9161	1.4706	44.0	97.8	80.9	192.6	26.6	2.37
French commercial oils, not	Max.	0.9183	1.4713	51.5	114.4	85.0	195.3	30.8	3.63
adulterated (61 samples).	Min.	0.9150	1.4699	40.7	90.4	79.0	190.5	23.7	0.45
	Avg.	0.9166	1.4708	45.1	100.1	81.3	193.0	27.3	1.59

* Rise of temperature with water = 45°.   † Next lower = 3.51 per cent.

genuine product or a mixture of a pure oil of low iodine number with an adulterant that would tend to raise the value. In the former case, however, the oil would probably show also a high specific gravity, refractive index, and specific temperature reaction, thus making the standard of comparison more rigorous and tending better to exclude adulterated samples. The California oils, in general, show higher iodine numbers and lower melting points of the fatty acids than do the French and Italian oils.

The usual range of "constants" to be expected may be tabulated as follows:

Specific gravity, 15.6°/15.6°............................ 0.915–0.917
Refractive index, 15.5°................................. 1.4705–1.4715
Iodine number.. 80–87
Saponification number.................................. 188–194
Iodine number of acids................................. 93–101
Titer test (°C.)....................................... 18–21
Maumené value (°C.).................................... 44–46
Free fatty acids (per cent)............................ 0.3–1.5
Unsaponifiable matter (per cent)....................... 0.8–1.5
Melting point of sterols (°C.)......................... 135–135.5
Melting point of acetates (°C.)........................ 120.3–120.7

Detection of Possible Adulterants of Olive Oil. 1. *Cottonseed Oil.*—The edible grades of cottonseed oil, varying in color from nearly colorless to a light straw, are employed in considerable quantities, either mixed with olive oil or as a substitute under the name of "salad oil." In order that the oil shall not become turbid in cold weather, much of it is chilled, and the solid "stearin" filtered off and pressed. The cottonseed stearin thus obtained is used in lard and butter substitutes (see page 226). The term "stearin" is a misnomer, since practically no stearic acid is present in cottonseed oil, the solid fatty acids consisting mainly of palmitic acid.

In general, the addition of cottonseed oil to olive oil would raise the specific gravity, iodine number, specific temperature reaction, the melting point of the fatty acids and titer test. Of these the last is especially characteristic (see Table 27, page 199). Some of these might fail to show the adulteration if a third oil, say lard oil, were skillfully added also, but, if the examination were reasonably complete, such a mixture could hardly escape detection.

Qualitative Test.—Cottonseed oil gives several fairly definite and characteristic color reactions, which are extremely useful as confirmatory tests or to differentiate the results of the quantitative examination as in the mixture cited above. They should, however, not be considered absolutely decisive or relied on to the exclusion of the analytical constants. Of these the two most useful are given below.

Halphen Test.[1]—To a 1 per cent solution of sulphur in carbon bisulphide add an equal volume of amyl alcohol. Mix 3 to 5 cc. of the reagent and an equal volume of the oil to be tested in a test tube and heat in a bath of boiling water, or better in boiling saturated brine, giving a temperature of about 105°C. Continue the heating for 2 hours unless a color develops sooner. A reddish color is developed in the presence of cottonseed oil, being more intense and more rapidly produced the greater the proportion of cottonseed oil present.

By comparing the color with that given by known mixtures of olive and cottonseed oils under identical conditions it is possible to determine approximately the percentage of the latter present.

[1] HALPHEN: *J. pharm. chim.*, **1897**, 390.

Notes.—This test is undoubtedly the most delicate for cottonseed oil, as little as 1 per cent being detected easily within the limitations given below. Cottonseed oil from different sources varies somewhat as regards the intensity of the reaction, but it has never been reported as given by any genuine olive oil.

The exact nature of the substance producing the red color is not definitely known[1] but it is present in very small amount and is destroyed or removed with relative ease.

The production of a red color cannot be taken as conclusive evidence of the presence of cottonseed oil since kapok oil, from the seeds of a tropical tree related to the cotton plant, and baobab oil give a similar reaction, the latter even stronger than cottonseed oil. Milliau[2] recommends the following procedure to distinguish them:

Saponify the oil, set free the fatty acids, wash, and dry. Mix 5 cc. of the melted acids with 5 cc. of a 1 per cent solution of silver nitrate in absolute alcohol, shake, and allow to stand without heating. The presence of 1 per cent or more of kapok or baobab oil causes a dark-brown color in 20 minutes; whereas with cottonseed oil there is no reducing action unless the mixture is warmed.

It has also been shown that lard, lard oil, or butter fat obtained from animals that have been fed on cottonseed meal will give the Halphen test even though no cottonseed oil has been added directly.

On the other hand, a negative test is no proof of the absence of cottonseed oil. Oil that is very old and rancid or has been heated to 250°C. for 10 to 20 minutes, or even to 200°C. for 1 hour, no longer gives the Halphen reaction. It is true that the heating of the oil gives it a disagreeable taste so that it would be less likely to be mixed with an edible oil. Hydrogenated oils no longer give the Halphen test. If then, the analysis points toward the presence of cottonseed oil and no positive result is obtained in the Halphen test, judgment must be based on the quantitative results only.

2. *Peanut Oil.*—Peanut oil (called also *arachis oil*) occupies the same importance in European countries as an adulterant of

[1] See among others RAIKOW: *Chem.-Ztg.*, **1900**, 562, 583; **1902**, 10; GILL and DENISON: *J. Am. Chem. Soc.*, **1902**, 397; RUPP: *Z. Nahr.-Genussm.*, **1907**, 74.

[2] *Compt. rend.*, **1904**, 807.

olive oil that cottonseed oil does in this country. Apart from the fact that the refined oil, being practically colorless and tasteless, is admirably suited for admixture with olive oil, its analytical characteristics so clearly resemble those of olive oil that considerable quantities of it can be added to the latter without being detected by the usual quantitative figures. The iodine number is somewhat higher, but still a large proportion could be added to an olive oil of low iodine value and escape detection. The only successful method for showing its presence consists in the separation and identification of the characteristic arachidic acid, of which peanut oil contains approximately 5 per cent.

Detection of Arachidic Acid. Bellier's[1] Method.—This test as modified by Evers is a simple and fairly satisfactory one.

Method.—Measure 1 cc. of the oil into a small dry flask, add 5 cc. of 1.5N alcoholic potassium hydroxide and saponify by heating on a water bath for 5 minutes, using a reflux condenser to prevent loss of alcohol. Add 50 cc. of 70 per cent alcohol, then 0.8 cc. of hydrochloric acid (sp. gr. 1.16). Heat if necessary to dissolve any precipitate that may be formed, then cool the solution in water, stirring continuously with a thermometer, so that the temperature falls at the rate of about 1°C. per minute. Olive oil will not in general give a clouding or turbidity above 9°C. If a turbidity appears before this temperature is reached, it is presumptive evidence of the presence of peanut oil, which may be confirmed if desired by the separation and identification of arachidic acid as in the Renard test.

Notes.—The method depends, as does the Renard test, given below, on the relative insolubility of arachidic acid in cold alcohol as compared with its lower homologues, palmitic and stearic acids.

It is essential that the stirring be continuous, since local cooling will cause the premature formation of a turbidity. For this reason the cooling water should not rise above the level of the liquid in the flask.

The clouding temperature is best observed by looking through the liquid against a good light and noting the temperature at which a definite precipitate first appears. The point is quite sharp and can usually be determined within ± 0.25°C.

[1] *Ann. chim. anal.*, **1899**, 4; EVERS: *Analyst*, **1912**, 487; **1937**, 96.

Occasionally after acidification an oil gives a slight opalescence that is unaffected by warming. This may be disregarded, since it does not affect the true turbidity temperature. The table below shows the character of the results obtained with pure oils:

	Clouding point, °C.
Olive (23 samples)	5.5–8.5
Peanut (13 samples)	39.0–40.0
Rape	22.5
Sesame	15.
Cottonseed	13.
Corn	7.5
Olive + 25 per cent peanut (25 samples)	9.0–12.5

It will be evident from the table that, if a turbidity occurs above 9° and qualitative tests have shown the absence of the three interfering oils, it should be reasonable proof of the presence of peanut oil without the laborious isolation and identification of arachidic acid.

Renard Test.[1]—Weigh 20 grams of oil into an Erlenmeyer flask and boil with 200 cc. of alcoholic potash (40 grams of potassium hydroxide per liter of alcohol) until saponified. Neutralize with dilute acetic acid (sp. gr. 1.04), using phenolphthalein as an indicator, and pour gradually into a 500-cc. flask containing a boiling mixture of 100 cc. of water and 120 cc. of 20 per cent lead acetate solution.

Boil for 1 minute and then cool the mixture by immersing the flask in cold water, preferably ice water, whirling the flask from time to time so that the precipitated lead soaps will stick to its sides. When thoroughly cooled pour out the excess of lead acetate solution and wash the soap with cold water and then with 90 per cent alcohol, draining as thoroughly as possible. Add 200 cc. of ether, cork the flask, and allow to stand, shaking occasionally, until the soap is disintegrated, after which heat gently to boiling on a steam bath or electric heater, using a reflux condenser. (*Avoid the use of a flame!*) Boil for 5 minutes, then cool to 15 to 17°C. and let stand overnight in an ice chest.

Filter, wash the insoluble soaps thoroughly on the filter with ether, then wash them back into the flask with a stream of hot water acidified with hydrochloric acid. Add a considerable

[1] RENARD: *Compt. rend.*, **1871**, 1330; TOLMAN: U. S. Dept. Agr., *Bur. Chem. Bull.* **107**, p. 145; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 421.

excess of dilute hydrochloric acid and enough hot water to give a volume of about 250 cc., and heat on the water bath or over a small flame until the soap is entirely decomposed and the fatty acids separate as a clear oily layer. Frequent shaking will hasten the process, which usually requires about 1 hour. Nearly fill the flask with hot water, and when the fatty acids have entirely separated stand the flask in cold water until they form a solid cake. Remove the cake of fatty acids, drain, then return to the empty flask, fill up with hot water and repeat the process. After the second washing dissolve the cake of acids in 100 cc. of boiling 90 per cent (by volume) alcohol. Cool to 15°C. and keep at this temperature with frequent shaking as long as any acid continues to crystallize out.

Filter into a graduated cylinder, wash the crystals twice with 10 cc. of 90 per cent alcohol, noting the total volume of filtrate and washings, then wash several times with more dilute alcohol (70 per cent), in which arachidic acid is only very slightly soluble. Make a hole in the filter, wash off the precipitate with boiling absolute alcohol into a tared dish, evaporate on the water bath, dry, and weigh. Correct the weight for the arachidic acid that remains dissolved in the filtrate and washings by adding 0.0025 gram for each 10 cc. of 90 per cent alcohol collected in the cylinder if the crystallization were carried out at 15°C., and 0.0045 gram for each 10 cc. if done at 20°C. The approximate weight of peanut oil (although probably too low) is obtained by multiplying the weight of arachidic acid by 20. Determine the melting point of the arachidic acid obtained, using the capillary tube method, page 178.

Notes.—The melting point of the "arachidic acid" obtained, which is in reality a mixture of arachidic and lignoceric acids, is somewhat variable, depending upon the care with which the process has been carried out, but should not be appreciably below 71 to 73°C. The melting points of the pure acids are distinctly higher than this (77° for arachidic and 80.5° for lignoceric acid), the lowering being due to impurities.

It is essential to determine the melting point of the crystals, since cottonseed and lard oils have been found to give crystals resembling those of arachidic acid but having a considerably lower melting point.[1]

[1] TOLMAN and MUNSON: U. S. Dept. Agr., *Bur. Chem. Bull.* **77**, p. 35.

The separation of the lead soaps by ether (see page 172) is advantageous since in this way the acids of the oleic series, which interfere with the crystallization of the arachidic acid, are removed as their soluble lead salts, allowing the detection of as little as 5 or 10 per cent of peanut oil.

Kerr[1] has suggested a modification of the Renard method in which the method is shortened and the use of ether avoided by converting the fatty acids into their magnesium salts and separating them by their relative solubility in alcohol.

In testing the purity of olive oil contained in manufactured food products, allowance must be made for the fact that peanut oil, on account of the better flavor it is supposed to give, may be used during the process and a small portion may be present in the final product. Thus in olive oil from canned sardines, the presence of peanut oil in quantities not exceeding 5 per cent is not regarded as an adulteration, since the fish are frequently cooked in peanut oil, drained, and then packed in olive oil.

Peanut oil contains from 3.5 to 5.5 per cent of arachidic acid according to different observers. The only other oils that contain any appreciable amount are rape and mustard seed, and in these the quantity is too small to have much analytical significance. Olive oil, with the possible exception of some African oils, contains only a trace.

According to Tortelli and Ruggieri,[2] the amount of peanut oil may be determined approximately by noting the temperature at which the separation of arachidic acid begins, as shown in the following table:

Temperature at which crystals begin to form, °C.	Per cent. peanut oil
35–38	100
31–33	60
28–30	50
25–26	40
22–24	30
20.5–21.5	20
18–20	10
16–17	5

3. *Sesame Oil.*—Sesame oil is obtained from the seeds of the sesame plant, grown chiefly in India, Egypt, and the countries

[1] *Ind. Eng. Chem.*, **1916**, 904.
[2] *Chem.-Ztg.*, **1898**, 600.

bordering on the Mediterranean, and is prepared in large quantities in France as an edible oil. It is used either alone or mixed with other oils, and in certain European countries its use is compulsory in the manufacture of oleomargarine. Its pleasant flavor and freedom from odor make it a natural adulterant for the more expensive olive oil.

As in the case of cottonseed oil, the specific gravity, refractive index, Maumené number, and iodine number of sesame oil are distinctly higher than for olive oil and serve to indicate its presence when added in considerable quantities. Like cottonseed oil also, definite color reactions are characteristic of sesame oil and highly important analytically. Of these the best are the two following:

Baudouin's Test.[1]—Dissolve 0.1 gram of cane sugar in 10 cc. of hydrochloric acid (sp. gr. 1.20), add 10 cc. of the oil to be tested, shake thoroughly for 1 minute and allow to stand for 10 minutes. If 1 per cent or more of sesame oil is present the aqueous layer will be colored red. Carry out at the same time a check experiment on an oil of known purity.

Notes.—Some genuine olive oils, especially those from Tunis and Algeria, give a pink color that might be attributed to a small percentage of sesame oil, but comparison with a sample of genuine sesame oil will usually show the difference. This is said to be due to a coloring matter derived from the pulp of the olive and in doubtful cases the test should be made upon the liquid fatty acids rather than the oil itself, since the interfering substance does not pass into the acids. If a distinct quantity of sesame oil is present the red color will be observed in both the oily and aqueous layers.

The reaction is attributed to one of the constituents of the unsaponifiable part of the oil, a phenolic substance[2] to which the name "sesamol" has been given. Other substances, such as vanillin and oil of cloves, will give a similar reaction; hence caution should be used in applying the test to oil extracted from confections or food products of mixed composition. Also it has been noted, as in the case of the Halphen reaction for cottonseed oil, that lard oil or butter prepared from animals fed on sesame cake will give a positive, though faint, test for sesame oil.

[1] *Z. angew. Chem.*, **1892**, 509.
[2] KREIS: *Chem.-Ztg.*, **1903**, 316.

Villavecchia and Fabris Test.—Villavecchia and Fabris found that the active agent in the preceding test was the furfural produced by the action of the hydrochloric acid on the sucrose, and they have modified the procedure by substituting an alcoholic solution of furfural for the sugar.

To 10 cc. of the oil to be tested add 3 drops of a 2 per cent alcoholic solution of furfural and 10 cc. of strong hydrochloric acid, and shake the test tube containing the mixture for 30 seconds. Allow the mixture to stand 10 minutes, observe the color, add 10 cc. of water, shake, and again observe the color. If the crimson color disappears, sesame oil is not present.

Note.—Furfural itself gives a violet color with hydrochloric acid; hence only a very small amount should be used.

4. *Corn Oil.*—Corn oil (maize oil) is produced in large quantities from the germs of Indian corn, *Zea mays.* Although considerably cheaper than olive oil, it has not in the past been largely used to adulterate the latter on account of its objectionable flavor. The modern refined oil, however, is nearly odorless and tasteless, and hence a possible adulterant that should be taken into consideration in the analysis of salad oils.

By reference to the table on page 199, it will be seen that for corn oil the specific gravity, Maumené number, refractive index, and iodine number are all decidedly higher than for olive oil, the last three values being even higher than for cottonseed oil. The high Maumené number and iodine number would render an ordinary olive oil suspicious if even only 10 to 15 per cent of the adulterant were added. A mixture of corn oil with lard oil might be used which would simulate olive oil quite closely so far as the above constants are concerned, but with such a mixture the difference between the specific temperature reaction and the iodine number would still be much greater than with pure olive oil.[1] Further, the presence of the lard oil would be revealed by its characteristic odor when heated and possibly by the phytosteryl acetate test (see page 197), bearing in mind that, although small amounts of phytosterol are quite readily detected in the presence of much larger quantities of cholesterol, the detection of animal fats when mixed with those of vegetable origin is much more difficult unless the percentage of animal oil is large.

[1] SHERMAN: "Organic Analysis," 2d ed., p. 183.

With any except the most highly refined corn oil, its presence would be revealed by the characteristic "grainy" odor and taste. There is no specific color reaction for this oil, although Tolman and Munson[1] state that nitric acid (sp. gr. 1.37) when shaken with corn oil gives a peculiar red color that is quite different from the color given with cottonseed oil.   If this test is employed, however, it should be made strictly comparative, and the results should be interpreted with great caution.

5. *Poppyseed Oil.*—This oil is pressed from the seeds of the oriental or opium poppy, *Papaver somniferum.*   The best grades, having almost no odor and a pleasant flavor suggestive of almonds, have been largely used in Europe as a salad oil and to adulterate olive oil.

What was said on page 211, under corn oil, regarding the raising of most of the constants of olive oil by its addition, applies with even greater force to poppyseed oil.   The specific gravity, Maumené number, refractive index, and iodine number are all exceptionally high, and unless masked by the use of lard oil would serve to indicate the adulteration.

6. *Soybean Oil.*—This oil is obtained from the seeds of the soy or soya bean, long cultivated in China, Japan, and Manchuria as a food material of diverse uses and now produced in the United States by the millions of pounds, largely for its oil content.   The oil is a drying oil and finds uses in soap making and, after suitable treatment, in paints, linoleum, etc., usually mixed with linseed oil.   The refined oil is used as a salad oil and in the manufacture of oleomargarine and mayonnaise.

The statements made under poppyseed oil regarding its effect on the constants of olive oil can be applied equally well to soybean oil, it being of the same character.

There is, unfortunately, no definite qualitative test for soybean oil, several that have been used more or less satisfactorily for differentiating the crude oil from linseed or tung oil having little value when applied to the edible grades.

7. *Tea-seed Oil.*—This oil is obtained from several species of tea plant, closely related to the tea grown for its leaves and used as a beverage, but cultivated in China and Japan especially for their oil content.   The refined oil is used as an edible oil and has found quite extensive use as an adulterant of olive oil, which it

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **77**, p. 37.

closely resembles in its analytical constants, so much so in fact that these tests are not sufficient to distinguish the two. There is apparently, however, a difference in the character of the sterols of the unsaponifiable matter. Of the various tests proposed on this basis the most reliable is that of Fitelson,[1] which has been tested satisfactorily on 284 olive oils and 55 samples of teaseed oil, including with both oils all grades, crude and refined.

Fitelson Test.—Measure into a test tube exactly 0.8 cc. of acetic anhydride, 1.5 cc. of chloroform, and 0.2 cc. of concentrated sulphuric acid. Mix and cool to room temperature. Add 7 drops of the oil to be tested directly to the reagents, mix, and cool again. (To measure the 7 drops of oil use glass tubing, 4 mm. outside diameter and approximately 2 mm. inside diameter. These 7 drops should weigh approximately 0.22 gram.) If the solution of oil in the reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each addition until a clear solution is suddenly formed. Appreciable deviations from these quantities, particularly in the sulphuric acid, cause distinct variations in color intensities. Since the mixed reagent deteriorates slowly, do not mix in advance of testing.

After the test tube and contents have remained at room temperature for 5 minutes, note the color produced. Tea-seed oil will show a deep green by reflected light and brown by transmitted light. Olive oil will show a green color by reflected and transmitted light, occasionally exhibiting a faint fluorescence. Add 10 cc. of anhydrous ethyl ether from a graduated cylinder and mix immediately by inverting once. Tea-seed oil will show a brown color changing to an intense red within a minute or so. This red color reaches a maximum and then fades slowly within a period of a few minutes. Olive oil forms an initial green color on addition of the ether. This color fades slowly to a brown gray, occasionally passing through a faint pink stage. Both olive and tea-seed oil will eventually fade to a permanent light-brown color. Mixtures of tea-seed oil and olive oil show the characteristic tea-seed oil colors proportional in intensity to the quantity of tea-seed oil present.

Note.—No other edible oil shows the deep red color with ethyl ether, all of the oils mentioned in connection with the adultera-

[1] *J. Assoc. Off. Agr. Chem.*, **1936**, 493; **1937**, 419.

tion of olive oil giving negative results, although some olive oils show a faint tinge of pink before fading to the final light brown. No color is produced by the free fatty acids from these oils. When the test is carried out at room temperature, the red color reaches its maximum intensity in about 1 minute after the addition of the ether, and if the mixture is cooled in ice water the maximum colors will be stable for about 5 minutes, permitting comparison with known tea-seed–olive oil mixtures tested simultaneously.

8. *Lard Oil.*—The liquid portion or olein of lard, obtained by subjecting it to hydraulic pressure, is sold as *lard oil*. The winter-pressed oil, especially if made from edible grades of lard, and well refined, is entirely free from the odor and taste of lard, being a bland oil, nearly colorless to light yellow, and admirably suited for the adulteration of olive oil. As much as 70 per cent has been reported in supposedly high-grade olive oil.

Further, analytically it much resembles olive oil, the specific gravity, refractive index, Maumené number, and saponification number being practically identical with the values given by olive oil. Its iodine number is decidedly lower than for olive oil although for purposes of adulteration this can hardly be regarded as a disadvantage, enabling the oil to be added in large proportion to olive oils of high iodine value or to be mixed with other oils as cottonseed or peanut, thus lowering the iodine number of the mixture to a point corresponding to that of olive oil.

For the detection of lard oil reliance must be placed chiefly upon the odor, the high melting point of the fatty acids and the presence of cholesterol.

Although lard oil carefully refined has very little odor when cold, it is intensified on heating the sample, and in many cases will serve for its detection if present in appreciable quantity: The analyst must of course first familiarize himself with the odor of lard oil itself, which is very similar to that of heated lard, and should experiment with mixtures of olive and lard oils, before drawing conclusions from the odor of an unknown sample.

The melting point of the mixed fatty acids, separated and determined as described on pages 193 and 178, is much higher than with olive oil, 21 to 30°C. in one case and 33 to 38°C. in the other. This difference, however, loses some of its analytical

value, since an olive oil of high iodine number, which is the kind of olive oil with which lard oil would be most likely mixed, would naturally have at the same time a low melting point for the fatty acids, so that a considerable proportion of lard oil could be added without making the melting point abnormal.

In cases of doubt it may be necessary to show the presence of cholesterol, this being characteristic of the animal oils as phytosterol is of the vegetable oils. Working details of the procedure, together with many helpful hints, will be found in Lewkowitsch-Warburton's "Oils, Fats and Waxes," Vol. II.

9. *Fish Oil.*—The effect that might be produced on the constants of olive oil by an admixture of fish oil should be given consideration for two reasons. In the first place, fish oil, especially menhaden oil, is said to be considerably employed as an adulterant, and second, because many varieties of small fish, as sardines, are preserved in olive oil. The olive oil in this case will always contain a proportion of the body oil of the fish, and some of its constants will be distinctly altered.

Menhaden oil, which may be taken as a type of the fish body oils, would be easily detected if added to olive oil in any large quantity, since its specific gravity, refractive index, Maumené number, and iodine number are all so much higher than those of olive oil. It would of course be much harder to find if lard oil were added at the same time. For the same reason higher constants must be expected in the case of olive oil taken from cans of preserved fish.

In addition to the raising of the constants, fish oil would in many cases be identified specifically by its characteristic "fishy" odor, which is more pronounced upon heating. Fish oils contain also cholesterol, which can be separated and identified by its crystalline form or by the melting point of its acetyl ester (see Lard Oil above). More easily carried out is the test given below.

Insoluble Bromide Test. *Qualitative.*[1]—Dissolve in a test tube about 6 grams of the oil in 12 cc. of a mixture of equal parts of chloroform and glacial acetic acid. Add bromine drop by drop until a slight excess is indicated by the color, keeping the solution cool by immersing the tube in a bath of water at about

[1] EISENSCHIML and COPTHORNE: *Ind. Eng. Chem.*, **1910**, 43; U. S. Dept. Agr., *Bur. Chem. Bull.* **137**, p. 87.

20°C. Allow to stand 15 minutes or more and then place the test tube in boiling water. If only vegetable oils are present, the solution will become perfectly clear, while fish oils will remain cloudy or contain a precipitate due to the presence of insoluble bromides.

Notes.—The method depends on the fact that the bromides of the unsaturated acids of vegetable oils, mainly oleic and linoleic acids, are completely soluble in the boiling mixture of chloroform and acetic acid, while the bromides of the somewhat different acids of fish oil remain undissolved (see also the notes under the "Hexabromide Test" on page 217).

Any cloudiness or precipitate obtained in the cold, before the solution has been heated, should be disregarded, since the method depends absolutely upon the behavior of the bromides in the hot solution. If the oil is absolutely clear at the beginning of the test, it will remain so at the boiling temperature, and any turbidity can be taken as indicative of fish oil. Five per cent of fish oil in olive oil can be detected with certainty.

Quantitative Determination. The "Hexabromide Test."[1]—Dissolve 1 to 2 grams of the oil in 40 cc. of ether to which 2 to 3 cc. of glacial acetic acid have been added, cool the solution to 5°C., and add bromine drop by drop from a pipette until a permanent brown color remains. Allow the solution to stand packed in crushed ice and in an ice box at least 3 hours, filter through a Gooch crucible, keeping the main bulk of the precipitate from the filter until the end, wash four times with ice cold ether, dry the precipitate in the water oven and weigh.

If a suitable centrifuge is available[2] it will be of advantage in removing the difficulties that attend the filtration of the gummy precipitate of insoluble bromides from the very volatile solvent. The process may be carried out in light test tubes, which can be whirled in the centrifuge and the separated and washed bromides dried and weighed directly in the tubes.

Notes.—The di- and tetrabromides, as would be formed from oleic and linoleic acids, are soluble in ether; the hexa- and octobromides of linolenic and clupanodonic acids are almost insoluble in cold ether.

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[1] Hehner and Mitchell: *Analyst,* **1893,** 313; Tolman: *Ind. Eng. Chem.,* **1909,** 341.

[2] Tolman, *loc. cit.*

The test is not characteristic of fish oils in that it is given also by linseed oil, the reaction being due in the latter case to the unsaturated linolenic acid, $C_{18}H_{30}O_2$, while with the fish oils it is probably due to the presence of clupanodonic acid, $C_{18}H_{28}O_2$, of the still less saturated series $C_nH_{2n-8}O_2$. In this case the compound formed would be octobromstearin $C_3H_5(C_{18}H_{27}O_2Br_8)_3$, instead of the hexabromstearin, $C_3H_5(C_{18}H_{29}O_2Br_6)_3$. The fish oils can be distinguished from linseed in this test, however, by noting the behavior of the insoluble bromides on heating. The bromides from linseed oil melt at about 144°C. to a *clear liquid*, while those from fish oils decompose before melting, forming a dark mass.

The test is considerably more satisfactory but more difficult and tedious if made on the fatty acids rather than the oil itself, especially when applied to linseed oil.[1]

The weight of insoluble bromides obtained from fish oil averages about 50 per cent, so that from the determination an approximation of the amount of fish oil present may be obtained.

10. *Rape Oil.*—Rape-seed oil, often called also *colza oil*, is obtained from the seeds of various species of *Brassica*, belonging to the general family of the *Cruciferæ*. The commercial oil is pale yellow with a distinct odor and an acrid, unpleasant taste. The more highly refined oils have been used considerably as a salad oil or to mix with olive oil.

The best quantitative reaction for the detection of rape oil in olive oil is undoubtedly the saponification number. The other values, as specific gravity, refractive index, and iodine number, although somewhat higher, do not differ greatly from olive oil. But, owing to the large proportion of the glyceride of *erucic acid* present in rape oil, its saponification value is low, erucic acid being one of high molecular weight (see page 171). The average saponification value of rape oil is about 175, while the average value for olive oil is about 195, and an olive oil having a saponification value below 190 should be regarded as suspicious. Castor oil has also a low saponification value (see page 199), but a very high specific gravity. It is not a common adulterant of edible oils, but if necessary, the determination of its acetyl value, as detailed in more comprehensive works, would serve for its identification, this being extremely high. Rape oil

[1] Steele and Washburn: *Ind. Eng. Chem.*, **1920**, 52.

differs also from olive in having a distinctly higher refractive index as compared with the specific gravity. The addition of mineral oil to olive would lower the saponification number equally well, but would cause a high value for unsaponifiable matter.

According to Tolman and Munson,[1] the presence of sulphur compounds in the oils of the *Cruciferæ* furnishes a means for their detection. If the oils are saponified with alcoholic potash and stirred with a silver spoon, the silver will become blackened by the formation of a sulphide. Lewkowitsch, however, points out that the test loses much of its value because the cold-drawn rape oils of commerce are free from sulphur, although oils prepared by the extraction process with carbon disulphide may still retain some.

The color reactions for rape oil, including the test with rosaniline bisulphite,[2] are unreliable, having failed in the writer's experience to show the presence of rape oil with any certainty in known mixtures.

The main chemical reactions for rape oil depend upon the properties of erucic acid, which may be separated under definite conditions as its lead or magnesium salt. Details of the complicated procedures necessary will be found in the papers cited below.[3]

The remarks that have been made regarding rape oil apply to mustard-seed oil, which is very similar in its properties, belonging to the same group. Fortunately, however, it is almost never used as an adulterant of olive oil.

BUTTER

The fat of cow's milk, on account of its universal use, has been selected as a representative edible fat although, as will be shown later, it is not in several respects typical of edible fats in general. Furthermore, the fat is not ordinarily used in a state of considerable purity, as is the case with olive oil, but is always mixed with other constituents from the milk. For this reason the dis-

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **77**, p. 38.

[2] PALAS: *Ann. chim. anal.*, **1896**, 434.

[3] TORTELLI and FORTINI: *Chem.-Ztg.*, **1910**, 689; THOMAS and YU: *J. Am. Chem. Soc.*, **1923**, 113, 129.

cussion is divided into two parts: the analysis of the butter itself and the examination of the butter fat.

A. ANALYSIS OF BUTTER

By act of Congress, approved March 4, 1923, butter was defined and the following standard provided for it: "That for the purposes of the Food and Drugs Act of June 30, 1906, 'butter' shall be understood to mean the food product usually known as butter, and which is made exclusively from milk or cream, or both, with or without common salt, and with or without additional coloring matter, and containing not less than 80 per centum by weight of milk fat, all tolerances having been allowed for."

General Composition.—As indicated in the foregoing definition, butter consists mainly of the fat of milk, together with a small and varying percentage of water, salt, and curd, the latter being made up chiefly of the casein of the milk.

The composition of 351 samples of butter, mostly of English and European origin, is summarized by König[1] in the following table:

COMPOSITION OF BUTTER

	Water, per cent.	Fat, per cent.	Casein, per cent.	Lactose, per cent.	Ash, per cent.
Maximum	35.12	90.92	4.78	1.63	15.08
Minimum	4.15	69.96	0.19	0.05	0.02
Average	13.45	83.70	0.76	0.50	1.59

The extreme limits given in the above table would, however, be rarely met in ordinary practice. The usual grades of marketable butter will generally show: Water, 9.5 to 14.0 per cent; fat, 82.5 to 88 per cent; curd, 0.5 to 1.50 per cent; ash, 0.50 to 5.0 per cent, the higher ash figures being obtained in the case of salted butter.

The average of 645 samples of American butters was: Water, 13.87 per cent; fat, 82.47 per cent; curd, 1.15 per cent; ash (salt, etc.), 2.51 per cent.[2]

[1] "Chemie der menschlichen Nahrungs- und Genussmittel," 4th ed., Vol. I, p. 309.

[2] THOMPSON, SHAW, and NORTON: U. S. Dept. Agr., *Bur. of Animal Ind.*, *Bull.* **149.**

Forms of Adulteration.—Apart from the question of the substitution of some foreign fat for the whole or a portion of the butter fat, which will be discussed later, as well as the matter of "renovated" or "process" butter, pages 227 to 228, the adulterations practiced consist of the inclusion of an excessive amount of water, a deficiency in fat content, or the addition of milk or milk powder. Special preparations have been found on the market for the purpose of enabling the incorporation into the butter of abnormal quantities of water or of milk. The addition of artificial color, being allowed by special act of Congress, does not constitute an adulteration, although the use of color in oleomargarine and other butter substitutes, since it enables the fraudulent sale of these as butter, is restricted. Preservatives are occasionally added.

METHODS OF ANALYSIS

Preparation of the Sample.—If the sample for analysis is to be taken from a considerable quantity of butter, great care must be employed in sampling, because the butter is usually not homogeneous in composition and cannot be mixed by stirring. The best plan is to take a fairly large sample of 100 to 200 grams or more, melt it at a temperature of not over 50°C. in a jar or wide-mouthed, glass-stoppered bottle and mix by violent shaking. Then cool until sufficiently solid to prevent the separation of the fat and water, taking especial care to shake the sample thoroughly during the cooling.

Water.—Place 1.5 to 2.5 grams of butter in a dish with a flat bottom having a surface of at least 20 sq. cm. and dry to constant weight at the temperature of boiling water, weighings to be made at the end of each hour. Fifty-cubic centimeter beakers can be used for dishes. These come to constant weight in 3 to 4 hours and permit a ready transfer of the residue to the crucible for the fat determination.

Notes.—If preferred, the butter can be dried on clean, dry sand or asbestos, and if a round-bottomed dish has to be used some such absorbent material is necessary.

It is essential if the butter is dried directly that it should be in the form of a very thin layer, since otherwise the water will be covered by the lighter melted fat and evaporation be prevented.

Rapid methods for the determination of water in butter, suitable for dairymen or for the routine examination of large numbers of samples, have been devised by Patrick[1] and Gray.[2]

Fat.—If no absorbent material was used in the water determination, dissolve the dried butter in the dish or beaker by macerating it with 15 cc. of petroleum ether boiling below 65°C. Transfer the contents of the dish to a weighed Gooch crucible containing a thin mat of ignited asbestos and wash the dish and crucible with petroleum ether from a wash bottle until free from fat, using about 100 cc. The last 25 cc. should pass through the crucible without using any suction. To avoid the formation of a crust of salt and casein in the beaker, add to the dried residue a small portion of petroleum ether and rotate at once until the fat is practically all dissolved. Allow to stand 1 minute and then rotate and transfer the entire contents at once into the Gooch crucible. This manipulation will remove practically all the residue from the beaker; any small remainder is easily transferred by a rubber-tipped rod and petroleum ether. Dry the crucible and contents to constant weight at the temperature of boiling water. Repeat the washing with 25 cc. of petroleum ether, dry and weigh again to constant weight to make certain that all the fat has been removed. The increase in weight of the crucible subtracted from the weight of the dried butter gives the weight of fat.

Note.—If the determination of water was made with the help of an absorbent it is evident that the above method will not apply. In this case extract the dried residue from the water determination with several successive portions of petroleum ether, decanting carefully each time through a small filter or Gooch crucible. Collect the filtrate in a weighed flask, evaporate the ether and dry the fat to constant weight in the water oven, weighing at intervals of 1 hour. Prolonged heating should be avoided on account of the danger of oxidizing the fat. The method is not entirely satisfactory on account of the persistent retention of a slight amount of the fat by the absorbent material.

Casein or Curd.—Ignite cautiously the crucible containing the residue from the fat determination until the residue is white or nearly so and the weight constant. This is best done in the

[1] *J. Am. Chem. Soc.*, **1907**, 1126.
[2] U. S. Dept. Agr., *Bur. of Animal Ind.*, *Circ.* **100.**

muffle furnace at 550°C. The loss in weight is the curd. If a more exact determination of the casein is desired the nitrogen may be determined by the Kjeldahl process (see page 37) and the casein calculated by the factor 6.38.

Ash.—The residue remaining in the ignited crucible after the curd has been burned is the mineral matter or ash.

The more important of the foregoing determinations may be carried out somewhat more expeditiously on one weighed sample as follows:[1]

Gooch Crucible.—Prepare a Gooch crucible of about 30-cc. capacity with a 0.1-gram pad of asbestos and place thereon 20 grams of R. R. alundum, 90-mesh. (This is a crystalline alumina especially prepared for carbon determinations. It can be purchased ready for use from the Norton Company, Worcester, Mass., for a nominal sum.) After use, the crucible is prepared again for further use by igniting in a muffle, washing with water, and drying at 100 to 105°C.

Process.—Weigh accurately in the weighed, specially prepared Gooch crucible 1.0 to 1.5 grams of the prepared sample, dry for 2 hours at 100 to 105°C., cool, weigh, and calculate the percentage loss in weight as moisture. Extract the fat from the dried sample by placing the Gooch crucible in a closed-system extraction apparatus, as shown in Fig. 51, and extracting for 30 to 40 minutes with carbon tetrachloride. Adjust the heat so that the solvent drops into the crucible at the same rate that the crucible drains, and keep the crucible nearly full of the solvent. When the extraction is complete, remove most of the solvent remaining in the crucible by applying suction for a few seconds. Dry the crucible for 30 minutes at 100 to 105°C., cool, weigh, and calculate the percentage of non-fat solids. Calculate the percentage of fat by subtracting the sum of the percentages of moisture and non-fat solids from 100.

If it is desired to determine the salt, wash it out of the non-fat solids with water and titrate the aqueous solution with standard silver nitrate solution, using potassium chromate indicator.

Notes.—Gooch crucibles prepared as described will retain approximately 10 per cent of the weight of the absorbent material used without loss of fat during the drying period. Only sufficient asbestos to hold the absorbent material should be used,

[1] MITCHELL and ALFEND: *J. Assoc. Off. Agr. Chem.,* **1926,** 209; **1928,** 39.

as it appears to be impossible to wash all the fat out of the asbestos without using excessive quantities of solvent.

Salt.—Weigh 10 grams of butter in a small beaker, add 30 cc. of hot water, and when the fat is completely melted transfer the whole to a separatory funnel. Shake the mixture thoroughly, allow the fat to rise to the top, and draw off the water, taking care that none of the fat globules pass the stopcock. Repeat
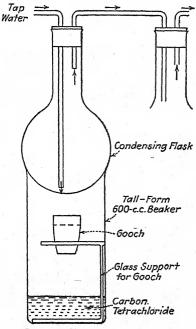
Fig. 51.—Extraction apparatus.

the operation ten times, using 20 cc. of water each time. Make the washings up to 250 cc., mix thoroughly, and titrate 25 cc. in a 6-in. porcelain dish, using $0.05N$ silver nitrate with potassium chromate as an indicator.

Preservatives. *Preparation of the Solution.*[1]—About 50 grams of butter are mixed with 25 cc. of chloroform in a separatory funnel, 100 cc. of dilute (0.1 per cent) sodium carbonate solution added and the whole mixed, avoiding violent shaking. After the separation of the layers, which may be facilitated by the use

[1] RICHMOND and HARRISON: *Analyst,* **1902,** 179; **1907,** 144.

of the centrifuge described on page 34, the aqueous layer is examined for preservatives. The principal preservatives to be sought are borax or boric acid, salicylic acid, sodium benzoate, and possibly fluorides. Use aliquot portions of the alkaline liquid and follow the methods described in the chapter on Preservatives, pages 103 to 120. The use of dilute sodium carbonate is to ensure the extraction of any free boric acid from the chloroform solution of the fat.

Artificial Colors.—The detection or identification of foreign colors in butter does not have the importance that it does in other foods, since the addition of color, being permitted by special legislation, does not constitute an adulteration. With oleomargarine and the various butter substitutes the question of artificial color is of more importance. Since the use of color enables the product to be sold more readily in substitution for butter, the addition of color is usually forbidden. In the United States under the Internal Revenue regulations a prohibitive tax of 10 cents a pound is put upon oleomargarine colored in imitation of butter. Of recent years the color most frequently employed has been palm oil, so-called "butter oils" sold for the purpose, consisting of cottonseed oil to which 2 to 5 per cent of the highly colored palm oil has been added. Cottonseed oil in which the natural color has been deepened by treatment with sulphur has also been used.

Special methods have been proposed to detect the addition of palm oil color.[1] Since, however, these tests must be carried out with absolute attention to the details of the procedure and require considerable experience with commercial oleomargarine in order to interpret the results properly, reference should be made to the original papers. Further, it has been stated that the same test is given by carotene and not necessarily by any added color.

Interpretation of Results.—Apart from the presence of preservatives, the most common form of adulteration of butter itself is in the incorporation of too large a proportion of water. This may be due to carelessness in making, too high a temperature in churning being a common cause, or to deliberate intent. Butter properly made should not contain more than 12 to 14 per cent of water at the most, and above 16 per cent usually constitutes

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[1] CRAMPTON and SIMONS: *J. Am. Chem. Soc.*, **1905**, 270; LEACH-WINTON: "Food Inspection and Analysis," 4th ed., p. 565.

grounds for prosecution for adulteration. Many prosecutions have been made by the Federal authorities for this alone.

The determination of the amount of curd may be of value in showing the addition of condensed milk or dried milk powder. If more than 1 per cent of curd is found it is probable that some milk product has been added, and confirmatory evidence will usually be found in the presence of an excess of water and of milk sugar.

The amount of salt has little significance from the standpoint of detecting adulteration, since the quantity added is dependent upon the taste of the consumer.

B. Examination of Butter Fat

Composition.—Butter fat differs markedly from other animal fats in the number of triglycerides that it contains, more than in any other fat, and in the relatively large proportion of the glycerides of acids of low molecular weight that is present.

Apart from this the characteristic feature of butter fat is the large proportion, fairly constant, of oleic and palmitic acids, and the smaller and less constant amount of stearic acid. Analyses by different observers vary greatly. Perhaps as good a summary as any is the following[1] made by modern methods and covering a wide range of locality and season for pasture-fed cows:

Acid	Per cent by weight
Butyric	2.6–3.5
Caproic	1.3–1.9
Caprylic	0.7–1.6
Capric	1.8–3.6
Lauric	3.2–5.7
Myristic	6.9–11.1
Palmitic	22.8–29.1
Stearic	6.5–12.5
Arachidic (?)	0.6–0.9
Oleic	31.3–41.3
Linoleic (?)	3.6–5.1

Other investigators have found a slightly different proportion of the various acids and evidence of the presence of mixed glycerides; the high percentage of linoleic acid has been ques-

[1] Hilditch: *Analyst*, **1937**, 250.

tioned, but, from the standpoint of the food analyst, the essential consideration is that the extremely high proportion of the glycerides of soluble and volatile fatty acids is characteristic of butter fat, and differentiates it from all others.

Forms of Adulteration.—Practically the only form of adulteration to be looked for in butter fat is the substitution of foreign fats. These may be substituted entirely, as in the case of oleomargarine, in which case the detection of the adulteration does not present any great difficulty, or they may be added in comparatively small amounts, even so little as 10 per cent. In the latter instance, on account of the great variation in composition of genuine butter, owing to different conditions under which the milk is produced and the butter manufactured, the problem is by no means an easy one.

The fats commonly employed are lard, beef fat, cottonseed oil, and coconut oil. These are not so often used singly but combined in various mixtures, which adds to the task of the analyst. Oleomargarine, for example, usually contains beef olein and lard, together with smaller amounts of cottonseed, peanut, or palm oils. Coconut oil, because of its relatively high content of soluble fatty acids, has been largely used in Europe as an ingredient of "butter oils." Such products, containing oleomargarine, cottonseed oil, and coconut oil, can be added in considerable quantities to butter fat without affecting most of the quantitative "constants" ordinarily determined. Of late years the use of these "nut butters" or "nut oleomargarines" has greatly increased. They are usually sold specifically as a butter substitute, uncolored, the purchaser being furnished a bit of color with each pound print. Different kinds of oil and either whole or skimmed milk are used in the manufacture of these products in this country. The oils most largely used are coconut and peanut oils that have been properly refined and deodorized. Other vegetable oils, as cottonseed, have been found occasionally, although not much at present. To obtain the desired consistency (softening and melting points), various proportions of hydrogenated oils are added, the amount depending in general upon the season of the year, more of the hardened fat being incorporated in the warm weather. The Federal authorities seized (1939) 90 tons of butter found to be adulterated with mineral oil.

Artificial preparations, containing tributyrin or triacetin in alcoholic solution, have even been added to make up for the decrease in volatile fatty acids caused by the use of animal fats.[1] The introduction in recent years of so-called "hardened oils" produced by the hydrogenation of vegetable oils, as mentioned above, brings a new possible method of adulteration to the consideration of the food analyst.

The flavor of butter is sometimes improved or counterfeited by the addition of diacetyl, $CH_3CO - COCH_3$. This occurs naturally up to 2 to 3 parts per million in butter fat, the flavor of butter being due largely to its production from acetylmethylcarbinol, $CH_3CHOHCOCH_3$, also found naturally in larger proportion in ripened cream, through oxidation or bacterial action. Larger amounts than stated are probably due to illegal addition.[2] (See also Vinegar, page 429.)

The substitution of renovated or "process" butter, although in a strict sense not the substitution of a foreign fat, is best taken up in connection with the detection of oleomargarine, hence is considered here rather than under the analysis of butter itself.

The raw material for the manufacture of renovated butter consists of butter that cannot be sold as such, either because of deterioration through rancidity or molding, or because, through carelessness on the part of the makers, it possesses an unattractive appearance or flavor. The chief recruiting ground for this material is the country grocery store. The fat, separated from the curd by melting and settling, is aerated to remove disagreeable odors and leave it nearly neutral. This is then emulsified with fresh milk that has been inoculated with a bacterial culture, and the whole is chilled, granulated, and churned. The butter is then worked and packed for market in the usual manner. The character of the product has much improved since the early days of the industry, the best grades now approximating the lower grades of creamery butter.

The Federal standard[3] reads: "Renovated Butter, Process Butter, is the clean, sound product made in semblance of butter from melted, clarified, or refined butter fat, without the addition

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[1] Lewkowitsch: "Oils, Fats and Waxes," 4th ed., Vol. II, p. 680.
[2] Barnicoat: *Analyst*, **1935**, 653.
[3] U. S. Dept. Agr., *Food Inspection Decision* **190**.

or use of any substance other than water, milk, cream, or salt, and contains, all tolerances provided for, less than sixteen per cent. (16.0 %) of water, and not less than eighty per cent. (80.0 %) of milk fat."

METHODS OF ANALYSIS

Analytical methods common to fats and oils in general have already been described on pages 174 to 197. The application of these to butter fat and the use of several that possess special value for the examination of butter fat will be considered here. Owing to the presence in butter fat of the lower members of the acetic series of fatty acids, the methods of greatest utility are in general those that afford a measure of (*a*) the mean molecular weight of the fatty acids; (*b*) the mean molecular weight of the soluble and insoluble acids; (*c*) the relative proportion of volatile and fixed fatty acids; and (*d*) the particular soluble or volatile acid present in largest proportion.

Preparation of the Sample.—Melt a considerable quantity, 50 to 100 grams, of the butter in a beaker on the water bath, taking care that the temperature does not exceed 60°C. After about 15 or 20 minutes the water, salt, and curd will have settled to the bottom, leaving the clear fat on top.[1] A quicker separation may be obtained by centrifuging the melted sample. Decant the layer of fat carefully through a filter paper or loose plug of cotton placed in a warm funnel. The filtering should be done in a warm place to prevent solidifying the melted fat, and the clear filtered sample should be preserved in a stoppered weighing beaker in the ice box until the analysis is completed.

Specific Gravity.—Determine this at $\frac{40°C.}{40°C.}$ following the method described on page 175.

Notes.—A minimum value of 0.905 at $\frac{40°C.}{40°C.}$ was formerly specified in the Federal standard for butter fat. The specific gravities of butter fat and some common adulterants determined at practically that temperature as tabulated by Lewkowitsch[2] are given on page 229.

[1] See also p. 243 in this connection.
[2] "Oils, Fats and Waxes," 4th ed., Vol. II, p. 714.

Fat	Specific gravity at 37.8°C. 37.8°C.
Butter (838 samples)	0.9100–0.9200
Mutton suet	0.9028
Beef suet	0.9037
Oleomargarine	0.9013–0.9038
Coconut oil	0.9100–0.9167
Lard	0.9050–0.9070

It is evident from the above figures that mixtures of coconut oil, for instance, with animal fats could be added in considerable quantities to butter fat, without being detected by the change in specific gravity. As a matter of fact, the specific-gravity determination would hardly be expected to yield information of much practical value. Although, as was pointed out on page 176, the fatty acids of low molecular weight have distinctly higher specific gravities, these acids are present in slight amount as compared with the proportion of insoluble fatty acids.

Melting Point.—Either of the methods described on pages 178 and 179, preferably the latter, may be used. Most of the fats used to adulterate butter fat have slightly higher melting points, but the test does not by any means afford as much information regarding adulteration as does the determination of refractive index, which may be carried out in a fraction of the time.

Refractive Index.—Follow the method outlined on page 177, making the determination at about 35°C. and calculating the results to either 25 or 40°C.

Notes.—The refractive index for butter at 25°C. is 1.4590 to 1.4620; for oleomargarine 1.4650 to 1.4700; for coconut oil 1.4520 to 1.4560. At 40°C. the corresponding values are: 1.4530 to 1.4560, 1.4590 to 1.4640, and 1.4460 to 1.4500.

As stated on page 177, the refractive index, on account of the ease and rapidity with which the determination can be carried out, has been largely used as a preliminary or sorting test to separate out the suspicious samples of fat or oil for more thorough examination. In the case of a complex fat like butter, however, the assumption must not be hastily made that a normal value for the refractive index necessarily implies the purity of the sample. It is comparatively easy to prepare a mixture of fats and oils that may be added in relatively large quantities to butter and leave the refractive index normal, being detected only by a more

thorough analysis. In this instance, as in others, coconut oil is of great service to the adulterator, since it has a lower index than butter fat, while beef fat and lard are higher.

Reichert-Meissl Number.[1]—The Reichert-Meissl number is *the number of cubic centimeters of 0.1N alkali required to neutralize the soluble volatile fatty acids distilled from 5 grams of fat.*

Process.—Weigh 5 grams (5.6 to 5.8 cc.) of the clear filtered fat into a 250-cc. round-bottomed flask, weighing to the nearest centigram only. Add 2 cc. of strong potassium hydroxide solution (1 + 1) and 10 cc. of 95 per cent alcohol. Connect the flask with a reflux condenser and heat on a steam bath so that the alcohol boils vigorously for 25 minutes. At the end of this time disconnect the flask and evaporate off the alcohol on a steam bath. After the complete removal of the alcohol, add 140 cc. of recently boiled distilled water that has been cooled to about 50°C. Warm the flask on the steam bath until a clear solution of the soap is obtained. Cool the solution to about 60°C. and add 8 cc. of sulphuric acid (1 + 4) to set free the fatty acids. Drop two bits of pumice,[2] about the size of a pea, into the flask, again attach it to the reflux condenser, and heat on the steam bath until the fatty acids have melted to an oily layer floating on the top of the liquid. Quickly attach the flask to a spray trap (Fig. 29, page 40) and condenser for distilling.

Distill 110 cc. into a graduated flask in as nearly 30 minutes as possible. Thoroughly mix the distillate, pour the whole of it through a dry filter to remove insoluble volatile acids, and titrate 100 cc. of the mixed filtrate with 0.1N sodium hydroxide, using phenolphthalein as an indicator. Multiply the number of cubic centimeters of alkali used by eleven-tenths, and correct the reading also for any weight of fat greater or less than 5 grams.

For example, if 5.3 grams of butter fat is used and 100 cc. of the distillate require 27.4 cc. of 0.1N NaOH, 110 cc. would require $27.4 \times 1\frac{1}{10} = 30.14$ cc. Then $5.3 : 30.14 = 5 : x$; $x = 28.4$. x is the Reichert-Meissl number.

Notes.—The three methods, Reichert-Meissl, Polenske, and Kirschner, should be considered together because they are used for the same purpose, to distinguish between butter fat and other

[1] See first paragraph under Notes.
[2] This should have been previously heated to a red heat and dropped into water. It is kept under water until used. One-tenth gram of powdered pumice may be used if desired, but the solution tends to bump ────

fats in general and to differentiate between butter fat and coconut oil in particular. The first of these is a general estimation of the fatty acids volatile with steam and soluble in water, which comprise butyric, caproic, caprylic, and to a less degree capric, lauric, and myristic, the last two with some decomposition. The characteristic acid of butter is butyric, which is determined separately by the Kirschner process. The volatile acids less soluble in cold water than butyric are caprylic, capric, and lauric, which characterize coconut oil and are the basis of the Polenske method. By suitable provision at the beginning all three methods may be carried out on the same sample. The student will be well advised to read through all three methods in order to plan his work before beginning the determination of any one.

Care should be taken that pure reagents are employed. Potassium hydroxide nearly free from carbonate should be used and the solution protected from the carbon dioxide of the air. The alcohol should be free from acid and aldehyde, and in critical work a blank test should be made on the reagents.

The process as described does not recover all the volatile fatty acids, 10 per cent or more still remaining in the distillation flask. By working under constant conditions, however, a fairly definite portion, and hence comparable results, are obtained. This emphasizes the fact that the method must be strictly followed in its details. Even minor points, such as the weight of fat taken, have a distinct bearing on the result. For example, a butter with a Reichert-Meissl number of 27.6 gave 32.7 when 1 gram was used, and 25.2 when 10 grams were used for the determination.

The Reichert-Meissl method is by far the most delicate for showing the presence of animal fats in butter because the differences obtained by it are so much greater than with other methods. This is illustrated by the table given below in which several common determinations are recorded for butter and for beef fat.

Determination	Beef fat	Butter
Specific gravity	0.945	0.935
Melting point	43°C.	30°C.
Saponification value	196	227
Iodine number	40	35
Insoluble fatty acids	95.5	87.5
Reichert-Meissl number	0.3	28.8

As Lewkowitsch points out, the determination is of paramount importance because a mixture of fats cannot be made that will have a Reichert-Meissl number normal for butter without using large quantities of butter fat itself in the mixture. On the other hand, mixtures of coconut oil and beef fat can be made of the same refractive index, saponification value, specific gravity, or iodine number as genuine butter fat, so that, if any one of these were applied as a preliminary or "sorting out" test, any proportion of the mixture could be added to butter fat and the sample passed as genuine (see also page 229).

The Reichert-Meissl number for genuine butter varies considerably with the time of year, the food of the cow, and the period of lactation, so that it covers quite a range, values having been reported as low as 12 and as high as 40. The usual variation is from 24 to 34. Coconut oil has a Reichert-Meissl value of 6 to 8, while practically all other edible fats have a value less than 1.0. Porpoise and dolphin oils are not edible but have extremely high values, 40 to 90.

Polenske Number.[1]—The Polenske number is *the number of cubic centimeters of $0.1N$ alkali required to neutralize the insoluble volatile fatty acids distilled from 5 grams of fat.*

The principle on which the method is based is that the volatile acids of butter fat, consisting mainly of butyric acid, are largely soluble in water, a small portion only being insoluble. On the other hand, the volatile acids of coconut oil, for the detection of which the method is principally used, are largely insoluble. By titrating these two portions separately the difference between butter fat and coconut oil can be more readily made apparent.

The method can readily be carried out in conjunction with the determination of the Reichert-Meissl number, using for convenience the Leffman-Beam modification of the latter.[2] (If the method detailed on page 230 is followed, care must be taken to remove every trace of alcohol.) The combined method is as follows:

Method.—Weigh 5 grams of the filtered fat into a 300-cc. round-bottomed flask, add 20 cc. of glycerol-soda[3] solution and heat over

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[1] POLENSKE: *Arb. kaiserl. Gesundh.-amte,* **1904,** 545; *Z. Nahr.-Genussm.,* **1904,** 270.

[2] *Analyst,* **1891,** 153.

[3] Twenty cubic centimeters of sodium hydroxide solution (1 of NaOH to 1 of water) to 180 cc. of glycerol.

a flame or asbestos plate until complete saponification occurs, as shown by the mixture becoming perfectly clear, which ordinarily requires about 5 minutes. If foaming occurs, shake the flask gently. Add 135 cc. of recently boiled water, drop by drop at first, if necessary, to prevent foaming, then add 5 cc. of dilute sulphuric acid $(1 + 4)$ and a few fragments of pumice stone. (Heat small pieces to a white heat, plunge into water, and keep under water until used.) Distill without previously melting the fatty acids, using an apparatus of the exact dimensions shown in Fig. 52. Rest the flask on a piece of asbestos board having a hole 5 cm. in diameter in the center, and so regulate the flame as to collect 110 cc. of the distillate in as nearly 30 minutes as possible and to allow the distillate to drop into the receiving flask at a temperature not higher than 18 to 20°C.

When the distillation is complete, substitute for the receiving flask a 25-cc. cylinder to collect any drops that may fall after the flame has been removed. Mix the distillate without violent shaking, immerse the flask containing it almost completely in water at 15°C. for 15 minutes, filter the 110 cc. of distillate through a dry filter paper 9 cm. in diameter, to filter out the chilled insoluble acids, and titrate 100 cc. with $0.1N$ sodium hydroxide solution, using phenolphthalein as indicator. The pink color should remain unchanged for 2 or 3 minutes.
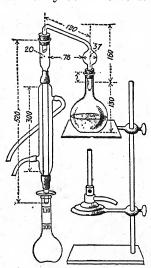
Fig. 52.—Polenske apparatus.

The Reichert-Meissl value is the number of cubic centimeters of $0.1N$ sodium hydroxide solution used times 1.1, after this result is corrected for the figure obtained in a blank determination. Save the titrated distillate, if desired for the Kirschner method (see page 235).

Remove the remainder of the soluble acids from the insoluble acids upon the filter paper by washing with three successive 15-cc. portions of water, previously passed through the condenser, the 25-cc. cylinder, and the 110-cc. receiving flask. Then dissolve the water-insoluble acids by passing successive 15-cc. por-

filter paper, each portion having previously passed through the condenser, the 25-cc. cylinder, and the 110-cc. receiving flask. Titrate the combined alcoholic washings with the standard sodium hydroxide solution, using phenolphthalein as indicator. The Polenske number equals the number of cubic centimeters of alkali solution required for the titration less the number required for a blank determination carried out in precisely the same way.

Notes.—The soap solution obtained should be perfectly clear and either colorless or faintly yellow. Old or rancid fats, which yield a brown soap solution, are not so well suited for the Polenske determination, although it should be said that old or rancid samples may still show a low Polenske value when the Reichert-Meissl number is abnormally high.[1]

The method has been studied by a number of observers since it was proposed by Polenske, and its value has been shown, but it is essential that the details of the process should actually be followed and that an apparatus of the form and dimensions shown in the figure be used. Important points are the size of the flask and the length of the condenser, since by increasing these varying results are obtained. The time of distillation should be kept as nearly as possible to that stated, and the flame should be so placed that only the bottom of the flask and not the asbestos support is heated. In order to test the apparatus as employed it may be advisable to carry out a determination on lard, which has a Polenske value of 0.5. Many valuable suggestions and detailed precautions to ensure good results will be found in the report of the Analytical Methods Committee of the English Society of Public Analysts.[2]

The method is perhaps most useful for the detection of coconut oil, for which it was originally devised, but is described at this point because, as suggested above, it can be readily carried out in connection with the determination of the Reichert-Meissl number, the Polenske apparatus serving for both determinations. The method may be still further employed for showing the presence of butter fat in mixtures containing also coconut oil by treatment of the neutralized distillate with silver sulphate, precipitating most of the volatile fatty acids with the exception of butyric, then redistilling and titrating the latter, as described

[1] In this connection see *Analyst,* **1931,** 515–518.
[2] *Analyst,* **1936,** 404–408.

below. Other methods, similar in principle but of more limited
scope, are described under the detection of coconut oil (page 245).
For the relative values to be expected in butter and its adulterants
see under coconut oil, page 247.

Kirschner Value.[1] *Method.*—To the titrated 100 cc. of the
Reichert-Meissl distillate, in a 200-cc. Erlenmeyer flask, add
0.3 gram of finely powdered silver sulphate. During the next
hour shake the mixture frequently, then filter and transfer
100 cc. of the filtrate into a 300-cc. flask. Add 10 cc. of dilute sul-
phuric acid (25 cc. of concentrated acid to 1,000 cc. of water), 35
cc. of water, and several small pieces of pumice stone. Distill
110 cc. in about 20 minutes, using the Polenske apparatus (Fig.
52). Titrate 100 cc. of the distillate with $0.1N$ sodium hydroxide
solution; and after correcting the number of cubic centimeters of
alkali solution used for the blank determination, calculate the
Kirschner value according to the following formula:

$$K = \frac{A \times 121(100 + B)}{10,000},$$ where A = the corrected Kirschner
titration, and B = the number of cubic centimeters of standard
alkali solution to neutralize the 100 cc. Reichert-Meissl distillate
from 5 grams of the fat $\left(\frac{11}{10} \times \frac{11}{10} \times \frac{1}{100} = \frac{121}{10,000}\right)$.

Notes.—Butter fat gives Kirschner values from 19 to 26; coco-
nut oil gives an average of 1.9 and palm-kernel oil 1.0, while the
majority of other fats and oils give values from 0.1 to 0.2.

The method for the determination of the Kirschner value
depends upon the solubility of silver butyrate in dilute solutions
of silver sulphate (the same would also be true of acids lower in
the series) and on the insolubility of the silver salts of acids
higher than butyric acid in the series. The chief use of the
method is to detect the presence of butter fat in admixture
with other fats and oils. Although palm-kernel and coconut
oils give appreciable Kirschner values, it should be noted that
neither of these products contains butyric or other acids lower
in the series; these results, however, do indicate the slight solu-
bility of the silver salts of caproic, caprylic, or capric acids.

In the interpretation of Kirschner values, it is suggested that in
the absence of coconut or palm-kernel oils or their stearins, only

[1] KIRSCHNER: *Z. Nahr.-Genussm.*, **1905**, 65; REVIS and BOLTON: *Analyst*,
1911, 333.

values above 0.5 should be considered as giving indications of the presence of butter fat, and the same applies to values above 2.6 in the case of fat mixtures containing coconut or similar oils. In ordinary circumstances the Reichert-Meissl and Polenske processes will be sufficient in examining butter fat. If the question is one of the presence or amount of butter fat, the Kirschner method should be employed as well.

Insoluble Fatty Acids (Hehner Number).—The Hehner number is *the percentage of insoluble fatty acids obtained from a fat or oil.*

Method.—Weigh out 2.0 to 3.0 grams of the oil or melted fat into a 500-cc. beaker, add 1 cc. of potassium hydroxide solution (1 + 1) and 20 cc. of 95 per cent alcohol. Cover the beaker with a watch glass and heat it on the steam bath until the liquid is clear and homogeneous. Evaporate off the alcohol on the steam bath and dissolve the soap in about 400 cc. of warm distilled water. When the soap is completely dissolved, add 10 cc. of hydrochloric acid (sp. gr. 1.12), and heat the beaker on the steam bath almost to boiling until a clear oily layer floats. Meanwhile dry a thick filter in a small covered beaker and weigh the whole. Allow the solution to cool until the fatty acids form a solid cake on top; filter the clear liquid, and finally bring the solid fatty acids upon the weighed filter. Wash the beaker and fatty acids thoroughly with cold water; then wash out the fatty acids adhering to the beaker with boiling water, which is poured through the filter, taking care that the filter is never more than two-thirds full. If the filter paper is of good texture and thoroughly wet beforehand, it will retain the fatty acids completely. If, however, oily particles are noticed in the filtrate, cool it by adding pieces of ice, remove the solidified particles with a glass rod, and transfer them to the filter. Cool the funnel by plunging it into cold water, remove the filter, place it in the beaker originally weighed, and dry at 100° to constant weight. The fatty acids should be heated about 1 hour at first, and then for periods of about 30 minutes, until the weight is constant within 2 mg. Prolonged drying may cause oxidation if large amounts of unsaturated acids are present.

Notes.—The residue remaining in the determination of saponification value (see page 181) may be used to estimate the insoluble fatty acids, if desired, by evaporating off the alcohol, dissolving the soap in water, and proceeding as described above.

There is some danger that small quantities of insoluble acids may be washed through the filter and thus the results be too low;

on the other hand, with butters containing unusually large amounts of lauric acid, this, being difficultly soluble in hot water, may not be entirely washed out, giving somewhat higher results.

The percentage of insoluble acids, which includes the small amount of unsaponifiable matter, varies from 86.5 to 88 in butter fat, while other fats and oils give values between 94.5 and 96.

Saponification Number.—Determine the saponification number as described on page 181.

Notes.—The average value for butter fat is 227, which is distinctly higher than for any other edible fat or oil with the exception of coconut oil and some samples of palm-nut oil. Lard and oleo products used in the preparation of butter substitutes have lower values than butter, but it is evident that mixtures of these with coconut oil can be prepared which will have the correct value for butter.

The determination is of greatest value in the analysis of butter fat when taken in connection with the Reichert-Meissl number. A high saponification value together with a low Reichert-Meissl value would be indicative of adulteration with coconut oil (see page 249).

Barium Value.[1]—The relation between the mean molecular weights of the soluble and insoluble fatty acids is one of the most valuable means of detecting modern forms of adulteration of butter fat with coconut oil and mixtures of similar fats, but the methods for actually separating the individual acids are difficult and tedious. The relation between the Reichert-Meissl and Polenske values answers well for the soluble and insoluble constituents of the volatile acids, and somewhat similar information may be gained regarding the total fatty acids by separating them as their soluble and insoluble barium salts.

Method.—Weigh out 5 grams of the filtered fat and saponify with 50 cc. of $0.5N$ alcoholic potassium hydroxide as described under the determination of the saponification number on page 181, making a blank determination as usual, in order to calculate the saponification value. After titrating with the standard acid, remove the alcohol as thoroughly as possible by boiling and blowing air into the flask. Dissolve the soap in hot water, cool to about 40°C., and make up to 250 cc. at that temperature.

[1] Avé-Lallemant: *Z. Nahr.-Genussm.*, **1907**, 317; Fritsche: *ibid.*, **1907**, 329; Revis and Bolton, in Allen's "Commercial Organic Analysis," 4th ed., Vol. II, p. 288.

Pipette off 100 cc. at the same temperature[1] into another 250-cc. flask, add 50 to 75 cc. of water, allow to stand on the water bath 5 minutes and add 50 cc. (measured exactly) of barium chloride solution (25 grams of crystallized barium chloride per liter). Allow the flask to remain on the water bath 15 minutes in order to coagulate the insoluble barium salts. Cool, make up to 250 cc. with water and filter off 200 cc. into a beaker. Heat this nearly to boiling, add 1 cc. of hydrochloric acid and 10 cc. of approximately normal sulphuric acid. Filter the barium sulphate on a Gooch crucible, wash free from chlorides with water, and finally with two 10-cc. portions of alcohol to remove any fatty acids still remaining. Dry at 100°C. to constant weight. Carry out a blank determination in the same way in order to determine the strength of the barium chloride solution.

Multiply the milligrams of barium sulphate found by five-fourths (to correct for the dilution) and calculate its equivalent in barium oxide. Subtract this from the barium oxide value of the barium chloride used, as determined in the blank. The difference is the barium oxide value of the fatty acids which form insoluble barium salts, and divided by 2, in order to calculate it to 1 gram of fat, gives the *insoluble barium value b*.

The saponification number, calculated to barium oxide, is the *total barium value a*, from which $a - b =$ the *soluble barium value c*. The most characteristic form of expressing the results, as shown in the Notes, is to calculate the numerical value for $b - (200 + c)$.

Example.—Five grams of butter fat require 40.60 cc. of 0.5N potassium hydroxide for saponification. The weight of barium sulphate obtained as above was 0.3347 gram and the blank was 0.9550 gram. Required the barium value.

$$\frac{40.60 \times 28.05}{5} = 227.8 = \text{saponification number.}$$

$$227.8 \times 1.367 = 311.4 = a.$$

$$955 \times \tfrac{5}{4} \times 0.6571 = 785 \text{ mg. BaO from blank.}$$

$$334.7 \times \tfrac{5}{4} \times 0.6571 = \frac{275 \text{ mg. BaO found.}}{510 \text{ mg. BaO in excess.}}$$

$$\frac{510}{2} = 255 \text{ mg. BaO in insoluble acids} = b.$$

[1] The regular laboratory apparatus may be used, since by having the temperature the same in both measurements no error is introduced.

$$a - b = 311.4 - 255 = 56.4 = c.$$
$$b - (200 + c) = -1.4.$$

Notes.—The value of the expression $b - (200 + c)$ varies considerably with pure butter fat but is almost invariably *negative*, while for the other edible oils and fats it is always *positive* and not less than $+ 39.0$. The addition of 10 per cent of coconut oil or beef fat to butter raises it to a positive value. One exceptional advantage of the method is that the effect of the simultaneous presence of lard and coconut oil is additive and not mutually destructive as in some other methods.

The method is not commonly used as a routine test and has not been so thoroughly tried as some others. The fundamental idea is excellent, but the method must be used carefully since errors of a few milligrams in weighing the barium sulphate will make quite a difference in the final result. It is perhaps best as confirmatory evidence in border-line cases.

Revis and Bolton[1] state that they have observed a few instances of mixtures of coconut oil and butter in which negative values were obtained for $b - (200 + c)$, but in such mixtures they have always found b to exceed 260.0, while in genuine butter fat b is always well below 260.0.

The following table, taken from their work, illustrates clearly the value of the method.

Sample	R. M. number	Pol. number	Sapon. number	Total BaO a	Insol. BaO b	Sol. BaO c	$b - (200 + c)$
Butter A	28.7	3.2	228.4	312.2	255.4	56.8	$- 1.4$
Butter A + 10 per cent coconut oil..........................	26.6	4.1	231.1	315.9	262.8	53.1	$+ 9.7$
Butter C	30.5	3.5	227.0	310.3	255.1	55.2	$- 0.1$
Butter C + 10 per cent coconut oil..........................	28.0	4.3	230.5	315.1	263.6	51.5	$+12.1$
Butter D ....✓......	30.8	2.9	224.8	307.3	252.8	54.5	$- 1.7$
Butter D + 10 per cent lard.....	27.7	2.4	221.8	303.2	254.6	48.6	$+ 6.6$

Other methods for detecting coconut oil are discussed on page 245.

Detection of Synthetic Triacetin.[2]—About 30 grams of the butter fat, whose Reichert-Meissl value has been determined, are

[1] *Loc. cit.*

[2] FINCKE: *Z. Nahr.-Genussm.*, **1908**, 666.

placed in a 600-cc. flask with 150 cc. of water and 150 cc. of 95 per cent alcohol. Some coarsely powdered pumice is added and the mixture boiled gently under a reflux condenser for 1 hour. After cooling, the fat is separated from the alcoholic layer and heated on the water bath until all water and alcohol are evaporated. On the dry fat the Reichert-Meissl number is again determined. With butter and other natural fats the Reichert-Meissl value remains practically unaltered, while in the presence of triacetin the second result is distinctly lower than the first.

Note.—The method is based on the solubility of triacetin in dilute alcohol. Mixtures of 4 to 6 per cent of triacetin and similar synthetic preparations with lard have been used to adulterate butter, since the Reichert-Meissl number of the mixture, being about 29, enables it to escape detection by the ordinary tests. The presence of 2 per cent of triacetin, according to Fincke, caused the Reichert-Meissl number to fall from 28.35 to 19.52 when the sample was treated as described above.

Interpretation of Results

The detection of foreign fats in butter should be classed among the most difficult problems of food analysis, and requires thorough physical and chemical examination in order to show the adulteration with certainty in all cases. The problem is in some ways a more difficult one than the examination of olive oil for adulterants because of the great natural variation that occurs in butter fat with manner of feeding, time of year, and period of lactation.

The following table shows the usual range of the constants for genuine butter fat:

Specific gravity, $\frac{40°C.}{40°C.}$	0.910–0.920
Refractive index, 40°C.	1.453–1.456
Melting point	28–34°C.
Iodine value	26–38
Saponification value	220–232
Reichert-Meissl number	23–30
Polenske value	1.7–2.9
Kirschner value	20–26
Titer	33–37°C.

In a general way it may be said that the presence of animal fats in any quantity would be shown by the saponification and

Reichert-Meissl numbers. Since the saponification numbers of the animal fats are usually below 200 and the Reichert-Meissl numbers less than 1.0, it is evident that the presence of animal fats (lard, beef, etc.) will lower both of these values. Vegetable oils would be shown by an increase in the iodine value, which with butter fat varies from 26 to 38, while olive oil has about 80 and the other fluid oils 100 or more. For conclusive evidence of the presence of a vegetable oil it may be necessary to make the phytosteryl acetate test (page 197), the presence of the oil being shown by a melting point of the acetate of 117°C. (corr.) and above.

Of all the general methods that have been described the Reichert-Meissl is undoubtedly the most useful as well as the most delicate. This is readily seen when it is recollected that in the saponification number the values obtained for butter and for oleomargarine differ by only about 15 per cent, and in the iodine number the value for oleomargarine is only about twice that for butter. With the Reichert-Meissl number, on the other hand, the value is 25 times as much in one case as in the other (see also page 242).

In a general way it may be said that the Reichert-Meissl-Polenske test should be applied to every suspected sample, and, when the value of the first named is above 24, and the Polenske value corresponds to this (page 247) the sample is probably genuine. If a value below 24 is found, the refractive index, saponification value, and iodine value should be determined.

It should be repeated, however, that no one test will show with certainty the addition of comparatively small amounts of mixtures of vegetable and animal oils as practiced at present. The refractive index, for example, was at first a good rapid test for the separation of genuine butter from its common adulterants, these all having a higher value. With the introduction of coconut oil all this was changed, since it is possible to prepare mixtures of animal fats and coconut oil covering the whole range of refractive indexes for pure butter; therefore as a sorting test the refractometer is now worse than useless.

The detection of several specific forms of adulteration is described in more detail beyond.

Detection of Oleomargarine.—The raw materials used in the manufacture of oleomargarine are largely "oleo oil," which is

mainly the olein of beef fat, neutral lard, and cottonseed oil, together with smaller amounts of butter and cream or milk. The natural effect of these upon the constants usually determined for butter fat would be to increase the percentage of insoluble fatty acids and the iodine number, and to lower the Reichert-Meissl number, saponification number, and specific gravity.

If the question involved is merely the distinction between the ordinary animal-fat oleomargarine and butter, it is comparatively simple, the constants noted above showing distinctly different values, as summarized in the following table.[1]

TABLE 29.—CONSTANTS OF BUTTER FAT AND OLEOMARGARINE

Substance	Sp. gr. at 100°C.	Hehner number	Saponification number	Reichert-Meissl number	Refractive index at 35°
Butter fat, maximum....	0.870	89.6	233	34.86	1.4578
Butter fat, minimum....	0.867	85.6	222	22.7	1.4557
Oleomargarine, maximum	0.862	95.5	203	5.5	1.4625
Oleomargarine, minimum	0.858	92.5	192	0.5	1.4613

When the case is so simple a single determination may be all that is needed to show the character of the fat in question. When oleomargarine is added in small amounts, however, the Reichert-Meissl method is the only satisfactory guide. The problem is made more complex, moreover, by the fact that oleomargarine as made at the present time may contain any of the following fats: coconut, palm-nut, beef tallow, lard, hydrogenated cottonseed oil, cottonseed stearin, as well as a small amount of butter.

There are no specific qualitative tests for oleomargarine, although the taste and odor are characteristic and enable experts to distinguish it from butter. In some European countries it is required that some oil easily detected by chemical tests, such as sesame oil, shall be added to oleomargarine. The "Spoon Test" (page 244) will serve to differentiate oleomargarine from butter, although it should be remembered that practically the same test is given by renovated butter.

Detection of Renovated Butter.—Since the fat of renovated butter is genuine butter fat, it is evident that the determination

[1] LEACH-WINTON: "Food Inspection and Analysis," 3d ed., p. 544.

of the usual "constants" will be of practically no help. Crampton[1] has shown that the changes in composition of butter fat caused by blowing air through it in the manufacture of renovated butter are less than the variations to be expected naturally in the fat itself. The only satisfactory methods for detecting renovated butter are those based on physical differences in the fat, caused by its being melted and then suddenly cooled in the process of manufacture, or on the character of the curd. Of these the most important are given below. There is no test that will show the addition of small quantities of renovated butter to genuine butter.

1. *Microscopic Examination.*—Pure, fresh butter is not ordinarily crystalline in structure. Butter that has been melted, however, and fats that have been liquefied and allowed to cool slowly show a distinct crystalline structure, especially by polarized light. If only fresh butter were sold, and all adulterants had been previously melted and slowly cooled, this method would be all that would be necessary for the detection of adulteration. As it is, however, it is most useful in making comparative examinations of samples that have been subjected to the same conditions.

It is, of course, necessary in the case of butter to be certain that it has been kept cool and not allowed to melt previous to making the microscopic examination.

If a bit of the fresh, unmelted sample, about the size of a pinhead, is taken from the center of the mass and pressed out on a slide by gentle pressure on the cover glass, it ought to show a fairly uniform field when examined with a 16-mm. objective.

In the case of renovated butter, however, there is a distinct difference to be noted in the appearance of the field. With genuine butter, the field is much more clear and free from opaque masses of curd than with renovated butter. When the field is examined by reflected light, turning the mirror so as not to pass light through the slide, these opaque masses in the case of renovated butter show strikingly as white masses against a dark background.

When examined also by polarized light, using a low-power objective and a selenite plate, pure butter shows no crystalline structure and an even color, while renovated butter shows a

[1] *J. Am. Chem. Soc.*, **1903**, 364.

mottled field with variegated colors caused by the double refraction of the fat crystals. The same effect is given by oleomargarine, and for the same cause. The test should be made on samples of genuine and renovated butter likewise in order to become familiar with the distinction before drawing definite conclusions regarding an unknown sample.

2. *"Spoon"* or *"Foam" Test.*[1]—Melt a piece of the sample as large as a small chestnut in an ordinary tablespoon or a small tin dish. Use a small flame and stir the melting fat with a splinter of wood (such as a match). Then increase the heat so that the fat shall boil briskly, and stir *thoroughly*, not neglecting the outer edges, several times during the boiling.

Oleomargarine and renovated butter boil noisily, usually sputtering as a mixture of grease and water does, and produce little or no foam. Genuine butter usually boils with much less noise and produces an abundance of foam, often rising over the sides of the dish or spoon when the latter is removed temporarily from the flame. The difference in regard to the foam is very marked.

Note also the appearance of the particles of curd after the boiling. With genuine butter these will be very small and finely divided, hardly noticeable in fact, whereas with oleomargarine and renovated butter the curd gathers in much larger masses or lumps.

Notes.—In genuine butter the curd is somewhat different in composition from that of renovated butter or oleomargarine in that it consists largely of the milk proteins that are insoluble in water, and hence accompany the separated cream. The curd of renovated butter or oleomargarine, on the other hand, comes from the proteins of the milk added directly in the process of manufacture, and consists mainly of coagulated casein. Hence its different appearance and behavior as regards foaming.

The crackling and sputtering of the fat in the case of oleomargarine and renovated butter are due to the fact that in the process of manufacture the melted fat is sprayed into ice water, and the cooled particles enclose some water.

Another effect, due to the difference in the curd of butter and of renovated butter, is seen in the appearance of the fat when the sample is melted on the water bath.[2] The curd of pure butter,

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[1] U. S. Dept. Agr., *Farmers' Bull.* **131**.
[2] HESS and DOOLITTLE: *J. Am. Chem. Soc.,* **1900**, 151.

being more gelatinous and cohesive, will readily settle out in a few minutes, leaving a fairly clear fat layer. The curd of renovated butter (and to a certain extent of oleomargarine also), being composed of the more granular casein, does not settle so readily but gives to the melted fat a turbid cloudy appearance, even after standing for several hours.

3. *The Waterhouse Test.*[1]—This test, although proposed originally as a test for distinguishing oleomargarine from butter, is also useful in detecting renovated butter. Crampton[2] finds it the most satisfactory of all the various tests.

Heat about 50 cc. of well-mixed sweet milk nearly to boiling and add 5 to 10 grams of the sample. Stir the mixture, preferably with a small wooden stick, until the fat is melted. Place the beaker in ice water, and when the fat begins to solidify, which usually requires about 10 minutes, stir thoroughly until the fat has hardened. Butter fat mixes with the milk, does not adhere to the wooden rod, and is slow to rise to the surface when the stirring is stopped. Oleomargarine is readily collected by the rod into a sticky lump, while renovated butter gathers in granular masses on the surface when the stirring is interrupted and does not readily form a compact lump.

The test is based on physical differences in the character of the fat that has been previously melted rather than on any difference in chemical constitution, as is shown by the fact that the test can be carried out nearly as well in water as in milk.

In all the above tests it must be remembered that similar results are given by oleomargarine, which must be differentiated from renovated butter by the usual chemical or physical tests on the fat. Further, the statement will bear repeating that no definite conclusion should be drawn from these simple tests until the student has become familiar with them by a comparative study of known samples.

Detection of Coconut Oil.—The third form of adulteration to be considered is by far the most difficult of detection. The adulterant is one that resembles butter fat in several of its constants more closely than any other fat used, and it is commonly added in relatively small amounts and often in mixtures with other

[1] PARSONS: *J. Am. Chem. Soc.*, **1901**, 200; PATRICK: U. S. Dept. Agr., *Bur. Chem. Bull.* **67**, p. 115.
[2] *J. Am. Chem. Soc.*, **1903**, 363.

fats. Considerable space is devoted to it here because its close resemblance to butter fat in its behavior offers an analytical problem of distinct interest, and because of its frequent use as a butter substitute.

The product in which it is most commonly found as an edible fat is the so-called "nut margarine" or "nut oleo," numerous analyses of which by Keister[1] are given in Table 30. Most of these contain peanut oil in addition, although one or two show the presence of cottonseed oil which is not commonly used at present.

TABLE 30.—ANALYSES OF NUT MARGARINES

						Constants of the fat					Qualitative tests	
Water, per cent	Fat, per cent	Curd, per cent	Ash (including salt) per cent	Sodium chloride, per cent	Free acid as oleic, per cent	Melting point, °C.	Reichert-Meissl number	Polenske number	Saponification number	Iodine number (Wijs)	Bellier's test for peanut oil	Halphen test for cottonseed oil
12.62	84.55	0.61	2.09	2.03	0.340	31 −32	5.73	9.16	237.4	13.54	Positive	Negative
10.04	85.79	1.25	2.53	2.43	0.290	42 −45	5.84	13.35	229.8	15.77	Positive	Negative
11.79	84.87	0.995	2.68	....	0.159	29 −29.5	4.93	11.86	228.2	21.68	Positive	Negative
13.55	83.49	1.42	1.56	....	0.142	24.6−24.8	5.44	13.12	252.1	12.50	Positive	Negative
10.97	82.62	1.52	4.96	....	0.087	25.2−25.7	4.94	13.11	232.4	20.57	Positive	Positive
10.63	85.90	0.93	2.51	....	0.170	25.6−26	5.18	13.65	237.0	19.50	Positive	Positive
13.06	82.18	0.56	4.13	4.07	0.180	25.5−26	5.00	10.18	238.8	21.94	Positive	Negative
12.83	82.96	2.18	2.03	2.00	0.408	26 −26.5	4.88	8.37	235.3	20.7	Positive	Negative
13.51	83.29	1.54	1.61	1.61	0.255	42 −43	4.29	7.13	228.8	26.45	Positive	Negative
13.62	80.72	2.51	3.25	3.23	0.280	44 −45	3.73	4.83	221.1	33.8	Positive	Positive
9.54	86.84	0.75	2.96	2.93	0.063	25 −25.5	5.59	10.95	237.79	24.67	Positive	Negative
11.11	84.06	2 11	2.77	2.77	0.260	....	4.19	9.40	227.1	17.90	Positive	Negative

As regards the analytical differences between coconut oil and butter fat, the fatty acids of coconut oil are made up as follows:[2]

	Per cent
Caproic acid	2
Caprylic acid	9
Capric acid	10
Lauric acid	45
Myristic acid	20
Palmitic acid	7
Stearic acid	5
Oleic acid	2

[1] *J. Assoc. Off. Agr. Chem.*, **1923**, 502.
[2] ELSDON: "Edible Oils and Fats."

These are admittedly somewhat rough figures, but they show the relative proportions of fatty acids.

Coconut oil thus differs from other vegetable oils and from the animal body fats in its high content of volatile fatty acids. It differs from butter fat in the large proportion of these fatty acids insoluble in water, and in the high percentage of caprylic acid (compare with the table on page 225). Upon the former fact is based its detection in butter fat by the Polenske method, and upon the latter its differentiation by various methods depending upon the separation of the acids into different groups according to the solubility of certain of their metallic salts. Note also that lauric and myristic acids predominate and that butyric acid is absent. There is little caproic acid, and the volatile acids are mainly caprylic and capric.

a. Polenske Number.—This method, the manipulation of which has already been described in connection with the Reichert-Meissl process (page 232), is the oldest and has been most widely used for the detection of coconut oil. The results with butter fat vary between 1.5 and 3.0, with palm oil between 8.5 and 11.0, and with coconut oil between 16.8 and 17.8. Since the Polenske number varies in butter in a general way with the Reichert-Meissl and Kirschner values the three have a somewhat definite relationship, as indicated in the table where the corresponding values to be expected are given.

TABLE 31.—COMPARISON OF REICHERT-MEISSL, POLENSKE, AND KIRSCHNER VALUES

Reichert-Meissl number	Polenske number	Kirschner number
23	1.6	20.0
24	1.7	20.5
25	1.8	20.9
26	2.0	21.8
27	2.4	23.3
28	2.6	24.0
29	2.9	24.8
30	3.0	25.0
31	3.2	25.8
32	3.5	26.4

By the addition of 10 per cent of coconut oil the Polenske number is usually increased about 1.0.

b. Hinks' Microscopic Method.[1]—Dissolve 5 cc. of the melted and filtered fat in 10 cc. of ether in a test tube which is then corked and placed in pounded ice for ½ hour. At the end of this time some of the solid glycerides will have settled out, leaving a clear ethereal layer. Filter rapidly through a plaited filter, and evaporate the ether (*away from a flame*). Transfer the residual fat to a test tube and boil it with three or four times its volume of 96 to 97 per cent alcohol. On allowing the solution to cool to room temperature most of the fat separates. Place the tube in water at 5°C. for 15 minutes, filter the clear alcoholic solution rapidly into another test tube, and place the latter in crushed ice in an ice box for 2 or 3 hours. Remove with a glass tube a portion of the flocculent precipitate that forms, place it on a slide, cover loosely with a cover glass, and examine it under the microscope, using a 4-mm. objective.

Since the crystals melt at about 5°C., the slide must be kept cold during the microscopical examination, which is readily done by keeping it on a Petri dish containing clear pieces of ice.

Notes.—The deposit in the case of butter will show under the microscope as round granular masses, whereas, with coconut oil, clusters of fine needlelike crystals will be observed. With mixtures, both forms will be seen, the coconut oil crystals sometimes appearing as separate clusters but more frequently attached to the butter fat granules. Typical fields are shown in Figs. 129 to 131, page 595, from Lewkowitsch's "Oils, Fats and Waxes."

It is important that the alcohol should be of the strength stated, since with weaker alcohol there is a tendency for the deposit from butter fat to be more or less crystalline.

Lard and beef fat give crystals somewhat similar to those obtained from coconut oil, but if these are present in quantities of 10 per cent or more, the characteristic granular appearance of the butter-fat masses is almost entirely obliterated.

The method requires some practice with the microscope to be carried out successfully, but after experience has been gained by a study of known samples, yields good results, as little as 5 per cent of coconut oil being detected by careful searching of the microscopic field.

c. The "Juckenack Difference."—Since in coconut oil the saponification number is higher and the Reichert-Meissl number

[1] HINKS: *Analyst*, **1907**, 160.

lower than in butter, the numerical difference between the two is a better indication of the presence of coconut oil than either one alone.[1]   For butter the value of the expression:

Reichert-Meissl number—(saponification number—200)

lies between + 4.25 and − 3.50, averaging about 0.0.   The corresponding value for coconut oil varies from − 40 to − 60.

The "barium value" has already been described (page 237) and its value in showing the presence of coconut oil pointed out. The Kirschner value (page 235) is of special significance in showing the presence of butter fat in admixture with coconut and other oils in oleomargarine.   In cases of extreme doubt final resort may be had to the phytosteryl acetate test (page· 197), but this requires considerable experience for satisfactory results.

It has been shown by several observers that if cows are fed with coconut cake or the residue from the oil presses, the butter will show practically all the changes in its constants that would be brought about by the addition of coconut oil directly.   Somewhat similar results are caused by feeding largely with turnips or turnip tops.   These practices, however, are not sufficiently common to prove a serious drawback to the analytical methods.

In general, it has been shown by numerous investigators that feeding with materials rich in vegetable oil, especially the press cake from oil seeds, such as cottonseed or sesame, will cause the absorption of a part of the oil into the milk and that the butter prepared from this source will have many of the characteristics of a mixture with the oil in question.   This in itself constitutes a strong argument for the phytosteryl acetate test, since careful experiments have shown conclusively that phytosterol is not transferred to either the milk or the body fat as a result of feeding with vegetable oils.

Detection of Hardened Oils.—A possible form of adulteration that has assumed much greater analytical importance is the addition to edible fats, as lard or butter, of the various "hardened" oils.   These are made by passing hydrogen through the heated oil in the presence of a suitable catalyzer, as finely divided metallic nickel or palladium.   The products thus made have as high a degree of edibility as the oils from which they are prepared, and, apart from any possible danger from the traces of

[1] Juckenack and Pasternack: *Z. Nahr.-Genussm.*, **1904,** 193.

nickel that may remain in the product, are probably unobjectionable as food material. The keeping qualities of the fat are distinctly improved, samples of hardened oils having been kept for 18 months exposed to damp air without showing any signs of rancidity. Some of the hardened oils have their appearance so changed that they resemble the edible animal fats and can hardly be distinguished from them as regards color, consistency, and flavor. For example, medium-hard peanut oil is so completely like neutral lard, and hardened whale oil so like mutton tallow that they cannot be distinguished by outward appearance from the genuine fats. Even so odorous a product as fish oil becomes changed into a solid odorless fat, thereby greatly increasing its possibilities as an adulterant.

These changes in the general properties of the oils by hydrogenation are accompanied by changes equally great in the analytical constants, especially those depending on the presence of unsaturated fatty acids. By the absorption of hydrogen such acids as oleic, linoleic, and linolenic are more or less completely transformed into stearic acid. In general this means a decrease in the iodine number and refractive index, and an increase in the melting and solidifying points. The saponification number remains practically unchanged. This change is a gradual one and can be stopped at any point desired, as illustrated in the following table[1] which shows the constants of the original oil (cottonseed) and of the hardened product at different stages.

	Original oil, clear liquid	Hardened oil		
		Solid particles floating in oil	Soft greasy solid	Brittle solid
Refractive index, 40°C..	1.4644	....	....	1.4578
Iodine value of fatty acids................	110	94	55	22
Titer test, °C..........	34.7	37	42.5	52.2
Saponification value.....	197	196	196	192

Such characteristic tests as the hexabromide test for fish oils (page 216) lose their value in the hydrogenated product on account of the change of the highly unsaturated acids to saturated

[1] KNAPP: *Analyst,* **1913,** 102.

acids, chiefly arachidic. The erucic acid of rape and mustard oils becomes behenic acid.

One method that indicates the presence of hardened oils is the determination of the amount of solid unsaturated fatty acids.[1] Natural edible oils other than rape or mustard will contain less than 3 per cent of solid unsaturated fatty acids except tallow, which may show 5 or 6 per cent. Rape oil contains about 50 per cent (erucic acid). Hardened oils usually show from 20 to 50 per cent of solid unsaturated acids.

The specific color reactions are variously affected, the Halphen test for cottonseed oil, for instance, failing to give positive results with the hardened oils, while the Baudouin reaction, on the other hand, is made even sharper by the hydrogen treatment. The studies of Kreis and Roth[2] indicate that of the general color reactions for showing the presence of seed oils in animal fats, only the Bellier test is suitable for use with hardened oils.

This test is as follows: Mix 5 cc. of the melted, filtered fat with 5 cc. of colorless nitric acid (sp. gr. 1.4) and 5 cc. of a cold saturated solution of resorcin in benzol, and shake the mixture violently for 5 seconds in a stout, glass-stoppered tube. If during the shaking or within the next 5 seconds, a red, violet, or green color is produced, seed oils are indicated. The later appearance of color should be disregarded. As in the case of other color reactions, experience should be gained by tests on known mixtures.

According to Bömer[3] phytosterol and cholesterol are unchanged by the hydrogenation, the acetyl esters obtained from hardened peanut, sesame, and cottonseed oils melting at 126 to 129°C., while the corresponding acetate from hardened whale oil had a melting point corresponding to cholesterol. The phytosteryl-acetate test (page 197) thus promises to be of great value in showing the presence of hardened vegetable oils in animal fats, its conclusions being more reliable, if the test is made with care, than the usual results of color reactions.

With some of the oils, as the fish oils, in which one of the chief results of the hydrogenation is the production of arachidic

[1] Cocks, Christian and Harding: *Analyst,* **1931,** 368.

[2] *Z. Nahr.-Genussm.,* **1913,** 81.

[3] *Z. Nahr.-Genussm.,* **1912,** 104.

acid, the presence of this as shown by the Renard or Bellier test (page 206) may be used as a means of identification.

It has been suggested[1] that tests for the traces of the nickel catalyst that remain in the product will be the best means of showing the presence of hardened oils. A delicate test for nickel is dimethylglyoxime, $CH_3C(NOH) \cdot C(NOH)CH_3$, which according to Fortini[2] is best used in alkaline solution. Fortini's reagent is prepared by mixing 0.5 gram of dimethylglyoxime, 5 cc. of 98 per cent alcohol, and 5 cc. of concentrated ammonium hydroxide, in the order given. This yields a clear, faintly yellowish liquid which may be kept in glass-stoppered bottles without change. The test is carried out as follows:

Heat 50 grams of the fat in a flask with 20 cc. of hydrochloric acid, with continued vigorous shaking. Allow the mixture to separate while hot and evaporate a portion of the acid solution to dryness in a porcelain dish. Heat the residue for a few moments in an oxidizing flame and add a drop of the above reagent. When nickel is present there will appear in a few seconds a rose color due to the reaction with the nickel oxide present on the surface of the metallic nickel.

This test is of value, of course, only in those cases where nickel rather than platinum or palladium has been used as a catalyzer, and, furthermore, Prall has pointed out that a few pure untreated oils may give a red color with dimethylglyoxime.

According to Kerr,[3] the latter difficulty may be avoided by destroying organic matter in the acid extract before testing for nickel.

Ten grams of the fat are heated on the steam bath with 10 cc. of dilute hydrochloric acid (sp. gr. 1.12), with frequent shaking, for 2 to 3 hours. The fat is then removed by filtering through a wet filter paper, the filtrate being received in a white porcelain dish. The filtrate is evaporated to dryness on the steam bath, 2 to 3 cc. of concentrated nitric acid being added, after it has been partly evaporated, to insure the destruction of all organic matter. The residue is dissolved in a few cubic centimeters of distilled water and a few drops of a 1 per cent solution of dimethylglyoxime in alcohol added. A few drops of dilute ammonia are then added.

[1] KNAPP: *Analyst*, **1913**, 102.
[2] *Chem.-Ztg.*, **1912**, 1461.
[3] *Ind. Eng. Chem.*, **1914**, 207.

The presence of nickel is shown by the appearance of the red color as above. The amount of nickel present may be estimated by comparing the color with that of a standard solution of a nickel salt.

Further information on the changes in analytical constants produced by the hydrogenation of oils will be found in Ellis (see References below).

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CHAPTER VI

CARBOHYDRATE FOODS

General Methods

Principal Carbohydrates.—Although thirty or more sugars have been isolated, those that are of direct importance to the food analyst are comparatively few in number. Important types of all the classes of carbohydrates, however, are among the common ones that we shall consider in this chapter.

They include:

Monosaccharides.—(Hexoses), Dextrose,[1] Levulose, Galactose; (Pentoses), Arabinose, Xylose.

Disaccharides.—Sucrose, Lactose, Maltose.

Trisaccharides.—Raffinose.

Polysaccharides.—Starch, Dextrin, Cellulose, Pentosans.

Monosaccharides.—*Dextrose* (*d*-glucose, grape sugar) is of wide natural distribution, being found as such in the blood of animals and in the juices of plants. It is found, also, in combined form as glucosides, for instance, salicin or amygdalin. The sugar is sweet to the taste and readily soluble in water or hot alcohol. The complex polysaccharides, of which starch and cellulose are typical, may be regarded as anhydride complexes which can be hydrolyzed into dextrose molecules. Most of these, as well as the disaccharides and the trisaccharide raffinose, yield dextrose upon hydrolysis with acids.

In the analysis of food products careful distinction should be made between dextrose or *d*-glucose and *commercial glucose*, which is not a natural product at all or even a definite chemical substance, but a variable mixture of dextrose, dextrin, and maltose, made by the incomplete hydrolysis of starch (usually corn) with dilute acid. A better name for the product is corn sirup or corn sugar.

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[1] On account of their simplicity and wide analytical use, the terms *dextrose* and *levulose* are used here instead of the *d*-glucose and *d*-fructose of Fischer's classification.

Levulose (*d*-fructose, fruit sugar) is usually present with dextrose in plant juices, very likely as the product of hydrolysis of sucrose by enzyme action, and exists particularly in honey, making up nearly 40 per cent of that product. Levulose is easily soluble in cold water or in hot alcohol. Its action on polarized light is strongly levorotatory, the degree of rotation being much affected by changes in temperature.

Galactose.—This sugar usually exists as a condensation product, *galactan*, a constituent of many gums, mucilages, hemicellulose, and other plant tissues. It has considerable analytical importance as a product of hydrolysis of lactose and of the galactans. Small quantities of copper-reducing matters present in some foods and reported as starch from the results of acid hydrolysis are undoubtedly galactan.

Pentoses.—Of the pentoses, or monosaccharides containing 5 carbon atoms ($C_5H_{10}O_5$), the only ones that need to be mentioned are arabinose and xylose, which are the products of hydrolysis of araban and xylan (see page 257).

The pentoses are characterized by strong reducing action on Fehling's solution and by their being converted into furfural when distilled with hydrochloric acid.

Disaccharides. *Sucrose.*—On account of its importance as a food product, sucrose is the best known of the sugars. It is found in many plants, in quantities ranging from 0.1 per cent to 25 per cent of the fresh material. The chief commercial sources are the juices of the sugar beet, sugar cane, and sugar maple. As prepared for consumption, it is one of the purest food products, ordinary granulated sugar being usually about 99.85 per cent pure sucrose, the remainder being mainly water. The hydrolysis products of 1 molecule of sucrose are 1 molecule of dextrose and 1 of levulose, the mixture being commonly known as *invert sugar*.

Lactose.—As sucrose is a sugar of distinctly vegetable origin, so lactose is derived entirely from the animal kingdom, being present in the milk of mammals in quantities varying ordinarily between 3 and 8 per cent. The sugar usually exists in the form of lactose monohydrate, $C_{12}H_{22}O_{11} + H_2O$, the water of crystallization being lost only when the sugar is heated to 130°C. By treating with acids under proper conditions, lactose is hydrolyzed into equal parts of dextrose and galactose,

the hydrolysis being, however, considerably more difficult than with sucrose. Lactose has practically no sweet taste and is much less soluble in water than cane sugar.

Maltose.—This sugar is ordinarily found in foods as the result of the action of amylases or starch-splitting enzymes such as occur in germinated barley or malt. It is found also as an intermediate product in the hydrolysis of starch by acids. Maltose is distinguished from the simple reducing sugars by its failure to reduce cupric acetate solution. When hydrolyzed by acids, 1 molecule of maltose yields 2 molecules of dextrose.

Trisaccharides. *Raffinose.*—This, the most studied and most widely distributed of the trisaccharides, is of interest to the food analyst mainly through its association with sucrose, especially in sugar-beet products. In some cases the methods for determining sucrose must be modified if raffinose is present, because it hydrolyzes with acids or certain combinations of enzymes into the monosaccharides, dextrose, levulose, and galactose. The reaction proceeds in stages, a disaccharide, melibiose, being an intermediate product. The first hydrolysis occurs even with weak acids or with invertase, hence the interference in sucrose determinations. Raffinose is strongly dextrorotatory, the specific rotation for the hydrate being +104.5. Like sucrose, it does not reduce Fehling's solution, indicating, as with sucrose, the absence of a functional aldehyde or ketone group.

Polysaccharides.—*Starch* ($C_6H_{10}O_5$) is the most important of the polysaccharides, being stored up as reserve food supply in roots, grains, and seeds, of which it may amount to 90 per cent or more of the dry material. In its natural unaltered form it appears as small white granules, which, as we have seen in Chap. II, have definite characteristic forms recognizable microscopically. The granules are insoluble in cold water but with hot water swell and disintegrate to form a paste. By treatment with acids, the action being greatly accelerated by heating, starch paste loses its colloidal characteristics and is gradually converted into a sirupy mixture of dextrin, maltose, and dextrose, finally being entirely changed into the latter.

Dextrin.—The usual method for the formation of dextrin is by the conversion of starch, which may be due to heating, to acids, or to enzyme action. Various forms of dextrin are obtained as intermediate products between starch and maltose in the

acid conversion of the former, the final product being dextrose. Dextrin, when prepared by diastatic starch conversion, or by heating starch with dilute nitric acid, and purified by precipitating with alcohol, gives a specific rotation of about $+195$.

Cellulose.—This is the most prominent single constituent of the vegetable kingdom, although it is rarely found in a pure condition, being present in the walls of cellular tissue combined with lignin and pentosans. Typical percentages of cellulose in the water-free material of various plant substances are tabulated by Browne[1] as follows:

Material (water free)	Approximate per cent of cellulose
Wood	60
Bark	40
Straw	40
Leaves	20
Seeds (including husks)	15
Roots, tubers, etc	10

Pentosans.—These are the parent substances or anhydrides of the pentose sugars, as arabinose and xylose, and are among the chief constituents of vegetable gums and tissues, araban in gum arabic, bran, corn stalks, etc., and xylan in wood gum. The latter is, next to cellulose, the most abundant of plant constituents, comprising from 15 to 30 per cent of the dry matter of straw, grass, and wood fibers. Boiling dilute acids readily dissolve and hydrolyze the pentosans into the corresponding pentoses, hence the necessity of removing them before determining starch by acid hydrolysis in an ordinary complex vegetable food.

Qualitative Tests for Sugars.—It is often desirable, before determining quantitatively the amount of sugar or other carbohydrates present in a food material, to learn what particular carbohydrates are present. The character of the food material itself will frequently give a fairly definite answer to the question. The sugars in milk chocolate, for example, would be expected to be sucrose and lactose. It should be borne in mind, however, in applying any qualitative tests, that the sugar or other carbohydrate should in general be separated so far as possible in a state of comparative purity, either through its solubility

[1] "Handbook of Sugar Analysis," p. 575.

or by other means, before applying the test. Some qualitative tests, as the well-known iodine reaction for starch, can be applied successfully in the presence of large amounts of other substances; with other tests, as the reducing action on copper solutions, quite erroneous conclusions may be drawn from the test unless interfering substances have been shown to be absent.

These qualitative tests may be *general*, applying to a group of sugars, or *specific* and characteristic of a particular sugar. A great many have been described, but the majority of them are applicable only to the pure substance and hence of limited use in the examination of foods.

General Qualitative Tests

The Molisch[1] or α-naphthol reaction is a widely used general test for carbohydrates. It is described by Mulliken[2] as follows:

Place about 5 mg. of the substance with 10 drops of water in a small, narrow test tube, and mix with 2 drops of a 10 per cent chloroform solution of α-naphthol. Allow 1 cc. of pure concentrated sulphuric acid to flow slowly down the side of the tube so as to form a layer beneath the aqueous solution. If a carbohydrate is present, a red ring will appear within a few seconds at the junction of the two layers. The color soon changes on standing or shaking, a very dark purple solution being formed. Shake and allow to stand for 1 or 2 minutes, then add 5 cc. of cold water. In the presence of a carbohydrate, a dull violet precipitate will be formed. Addition of an excess of strong ammonia will change the color to a rusty brown.

Note.—The reaction is assumed to be due to the formation of a condensation product between the α-naphthol and the furfural resulting from the decomposition of the carbohydrate.

Fehling's Copper Test for Reducing Sugars.—The reducing sugars may be detected by their action upon alkaline solutions of metallic salts. The monosaccharides, as well as lactose and maltose of the disaccharides that we have discussed, reduce alkaline solutions of metallic salts, such as copper or mercury, oxygen being withdrawn and the metal precipitated either as such or as a lower oxide. The metallic salt most commonly employed

[1] *Monatsh.*, **1888**, 198.

[2] "Identification of Pure Organic Compounds," Vol. I, p. 26.

is one of copper tartrate, known as Fehling's solution, and the method is described as a quantitative one on page 262. It should be noted in using this test as a qualitative one that many substances interfere with the test, either by preventing the precipitation of the cuprous oxide or by precipitating similar substances that might be mistaken for it. It is generally better to follow the precautions prescribed for the quantitative application and remove interfering substances with lead acetate; the excess of lead acetate is then precipitated with potassium oxalate and the clear filtrate tested by adding a few cubic centimeters of the solution to 10 cc. of Fehling's solution in a test tube and boiling 2 minutes. A brick-red precipitate of cuprous oxide will form if reducing sugars are present.

Barfoed's Test.—If the presence of a reducing sugar is shown by the previous test, and it is desired to distinguish lactose or maltose from a monosaccharide, a solution of cupric acetate as suggested by Barfoed[1] may be employed. This is reduced by the latter but not by the former. The reagent is prepared by dissolving 45 grams of neutral crystallized cupric acetate in 900 cc. of water, and filtering. To the filtrate is added 1.2 cc. of 50 per cent acetic acid and the solution diluted to 1 liter. A portion of the reagent, heated on the water bath, should show no reduction.

To make the test: To 5 cc. of the reagent, in a test tube, add 5 cc. of the solution to be tested and place in a boiling water bath for $3\frac{1}{2}$ minutes; examine for precipitated cuprous oxide, viewing the tube against a black background in a good light. If none is found, let the tube stand at room temperature for 5 to 10 minutes, pour out the liquid carefully through a filter and note if any traces of cuprous oxide remain adhering to the test tube or to the paper after rinsing with water.

Note.—Hinkel and Sherman found that the test would detect 0.4 mg. of dextrose in the presence of 20 mg. of lactose or maltose.

Osazone Reaction.—The qualitative test of most general application is the conversion of the sugar into its osazone. Those sugars that have the carbonyl group, *i.e.*, the reducing sugars, react with an excess of phenylhydrazine to form osazones. The reaction in the case of dextrose is a typical one:

[1] *Z. anal. Chem.*, **1873**, 27; HINKEL and SHERMAN: *J. Am. Chem. Soc.*, **1907**, 1744.

$$
\begin{array}{ccc}
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
| & & | \\
(\text{H}\overset{|}{\text{C}}\text{OH})_3 & & (\text{H}\overset{|}{\text{C}}\text{OH})_3 \\
| & & | \\
\text{H}\overset{|}{\text{C}}\text{OH} + 2\text{H}_2\text{N} - \text{NHC}_6\text{H}_5 \rightarrow & \overset{|}{\text{C}} = \text{N} - \text{NHC}_6\text{H}_5 + 2\text{H}_2\text{O} + \text{H}_2 \\
| & & | \\
\text{H}\overset{|}{\text{C}} = \text{O} & & \text{H}\overset{|}{\text{C}} = \text{N} - \text{NHC}_6\text{H}_5 \\
\text{Dextrose} \quad\quad \text{Phenylhydrazine} & & \text{Dextrosazone}
\end{array}
$$

To carry out the reaction, mix together 1 part of the sugar, 2 parts of phenylhydrazine hydrochloride, 3 parts of crystallized sodium acetate, and 20 parts of water in a test tube. Filter the solution if not perfectly clear, heat in a boiling water bath for 1½ hours, and allow it to cool.

A solution of phenylhydrazine acetate, prepared by adding glacial acetic acid to phenylhydrazine until it is just dissolved, may be used instead of the hydrochloride, if more convenient. In this case the sodium acetate is not necessary.

The osazone thus precipitated is of a yellowish color and more or less crystalline. The osazones of the monosaccharides separate from the hot solution; those of the disaccharides maltose and lactose, only after cooling. It is possible by washing with hot water to separate the two classes nearly completely. The osazone may be purified by filtering on a small paper, washing once with cold water, dissolving in the least possible amount of boiling 50 per cent alcohol, and filtering hot.

In order to identify the osazone thus obtained, its melting point should be determined by the capillary-tube method, as ordinarily used for solid organic compounds (see page 178). The principal limitation of the method is shown in the fact that the melting points of the osazones do not lie very far apart. Dextrose and levulose yield the same osazone, dextrosazone, melting at 204 to 205°; galactosazone melts at 191 to 196°; lactosazone at 200°, and maltosazone at 202 to 208°. It is possible to distinguish whether the osazone obtained is derived from a pentose, hexose, or disaccharide by determining its nitrogen content, or by noting the time required for osazone formation under definite conditions.[1] The osazones of the monosaccharides separate much more quickly than do those of the disaccharides. Under the conditions prescribed by Mulliken, the osazones of the common

[1] MAQUENNE: *Compt. rend.*, **1891**, 799; MULLIKEN: "Identification of Pure Organic Compounds," Vol. I, p. 32; SHERMAN and WILLIAMS: *J. Am. Chem. Soc.*, **1906**, 629.

monosaccharides precipitate from the hot solution in from 2 to 10 minutes, sucrose (due to slight inversion) in 30 minutes, maltose and lactose not at all. These figures were obtained, however, with the pure sugars. With mixtures, or in the presence of impurities, these times are greatly modified so that the method is of less value. A distinct help in this case would be a suitable polarizing microscope, since several of the osazones having nearly identical melting points are, for example, entirely different in the sign of elongation, a property that can be determined with certainty in a minute or two. For a discussion of the optical properties of the osazones, see Wright[1] and Morris.[2]

Special Group Tests

The Furfural Reaction.—The reaction of the pentoses or pentosans with hydrochloric acid, by which furfural is produced, finds considerable application in food analysis, a typical case of its qualitative value being in the detection of artificial invert sugar in honey, page 334. By carefully regulating the conditions, the yield of furfural may be made so nearly constant as to be utilized for the determination of the amount of pentosans, page 302. The reaction takes place through the splitting off of water:

$$\underset{\text{Pentose}}{C_5H_{10}O_5} \rightarrow \underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Water}}{3H_2O}$$

To carry out the qualitative test, boil about 5 grams of the material in an Erlenmeyer flask with hydrochloric acid (sp. gr. 1.06). Lay over the mouth of the flask filter paper soaked with anilin-acetate solution.[3] In the presence of furfural, the paper will turn to a brilliant red. Or the boiling with hydrochloric acid may be done in a distilling flask and the distillate tested with phloroglucin, which causes a dark-green precipitate of furfural-phloroglucid.

$$\underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Phloroglucin}}{C_6H_6O_3} \rightarrow \underset{\text{Furfural-phloroglucid}}{C_{11}H_8O_4} + H_2O$$

[1] *J. Am. Chem. Soc.*, **1916**, 1647.

[2] *Ibid.*, **1932**, 2843.

[3] Prepared by mixing in a test tube equal volumes of anilin and water and adding glacial acetic acid with constant shaking until the mixture becomes clear. Filter paper is moistened with the solution.

This is the reaction commonly used for the quantitative estimation of more than traces of furfural.

Note.—It should be remembered that many of the hexose carbohydrates, as sucrose or starch, as well as a few other substances, will yield furfural in this reaction, but the amounts are so small as not to interfere seriously with the test for pentoses.

The Orcinol Reaction.[1] *Reagent.*—Dissolve 1 gram of orcinol in 500 cc. of hydrochloric acid (sp. gr. 1.15) to which 20 drops of 10 per cent ferric chloride solution are added.

Test.—Heat 5 cc. of the reagent to boiling in a test tube, remove the solution from the flame, and add a few drops (never over 1 cc.) of the solution to be tested. If pentoses are present a vivid green color will develop almost immediately.

Note.—Various polyvalent phenols as phloroglucinol and orcinol will give color reactions with the pentoses in the presence of hydrochloric acid (see page 168). The test described is characteristic of pentoses, glycuronic acid, the only interfering substance, giving a similar color only upon prolonged heating.

The Resorcinol Reaction.[2]—This test is of value in distinguishing those sugars having a ketone group, more especially levulose, from the aldehydic sugars such as dextrose or lactose.

To make the test, add to 10 cc. of the sugar solution in a test tube an equal volume of 25 per cent hydrochloric acid and about 0.1 gram of resorcinol and heat gently over a small flame. If a ketose sugar is present, a fiery-red color will be developed.

Notes.—It is important that the test should be carried out as described, especially with respect to the amount of acid. If too much be used, other sugars will give pinkish solutions that might be mistaken for the more pronounced color of the ketoses.

The reason that the reaction is given on page 161 as a test for sucrose in cream is, of course, due to the fact that sucrose, when heated with acid, yields as one of its products levulose, a ketose sugar.

Quantitative Methods

The Determination of Reducing Sugars.—The fact that the reducing sugars, similar to aldehydes or ketones in general, possess the property of reducing certain alkaline metallic salts

[1] Bial: *Biochem. Z.*, **3**, 323; Tollens and Lefevre: *Ber.*, **40**, 4520.
[2] Seliwanoff: *Ber.*, **1887**, 181.

has already been noted under the qualitative use of Fehling's solution, page 258.

The reduction of the copper solution may be made quantitative for estimating the percentage of sugar by either (1) determining the volume of sugar solution required to precipitate a measured amount of the copper solution, or (2) by weighing or otherwise determining the cuprous oxide reduced from an excess of the copper reagent by a measured quantity of the sugar solution.

1. Volumetric Methods.—The original Fehling method was of this type, but they have fallen more or less into disrepute on account of the difficulty of determining the exact point of precipitation of the copper, this requiring in general the use of an inconvenient outside indicator. In recent years, however, among important researches that have been published on the volumetric estimation of reducing sugar, it has been pointed out by Lane and Eynon[1] that methylene blue may be used as an inside indicator in this reaction, it being rapidly decolorized by a slight excess of sugar, giving a readily discernible end point. The method has come into wide use as a rapid and convenient one, especially where many determinations of a quite similar nature are to be made.

Lane and Eynon Method. *Reagents.*—Use the copper sulphate and alkaline tartrate solutions as specified in the Munson and Walker method on page 266.

Standardization.—Pipette accurately 5 cc. of each solution, *a* and *b* into a 250-cc. Erlenmeyer flask. Prepare a standard solution of the pure sugar, conveniently either dextrose or inverted sucrose, of such concentration that more than 15 cc. and less than 50 cc. will be required to reduce all the copper. The number of cubic centimeters required (titer) may be calculated by $\dfrac{\text{factor}}{\text{mg. sugar in 1 cc.}} = $ titer. Add almost all the sugar solution needed to reduce all the copper, so that not more than 0.5 to 1 cc. is required later to complete the titration. Heat the cold mixture to boiling on a wire gauze and boil gently for 2 minutes, lowering the flame sufficiently to prevent bumping. Without removing from the flame add 2 to 5 drops of 1 per cent aqueous methylene blue solution and complete the titration within a total boiling

[1] *J. Soc. Chem. Ind.*, **1923**, 32T, 463T.

time of about 3 minutes by small additions of the sugar solution to decolorization of the indicator. A burette with a tip connected by rubber tubing and a pinch cock will be found more convenient than the customary all-glass one.

TABLE 32.—FACTORS FOR 10 CC. FEHLING'S SOLUTION TO BE USED IN CONNECTION WITH THE LANE-EYNON GENERAL VOLUMETRIC METHOD

Titer	No sucrose invert sugar	1 gram sucrose per 100 cc, invert sugar	5 grams sucrose per 100 cc, invert sugar	10 grams sucrose per 100 cc, invert sugar	25 grams sucrose per 100 cc, invert sugar	Dextrose	Levulose	Anhydrous maltose, $C_{12}H_{22}O_{11}$	Hydrated maltose $C_{12}H_{22}O_{11}.H_2O$	Anhydrous lactose, $C_{12}H_{22}O_{11}$	Hydrated lactose, $C_{12}H_{22}O_{11}.H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.7	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

Multiply the titer by the number of milligrams of sugar in 1 cc. of the standard solution to obtain the factor. Compare with the factor given in the table to determine the correction, if any,

to be applied in the determination. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents.

Determination.—If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 cc. of the Fehling's solution, measured as in the standardization, 15 cc. of the sugar solution and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar solution until only the faintest perceptible blue color remains. Then add 2 to 5 drops of the methylene blue and complete the titration by dropwise additions of sugar. In the table find the factor corresponding to the titer, applying the correction previously determined if necessary, and calculate the sugar by $\dfrac{\text{factor} \times 100}{\text{titer}} =$ mg. sugar in 100 cc.

For analyses of the highest precision repeat the titration, adding almost the whole of the sugar solution required to reduce all the copper, and proceed as described in the standardization.

Lane and Eynon[1] and Fitelson[2] have published correction tables for the "sucrose effect" in sucrose-lactose and sucrose-dextrose mixtures. The presence of sucrose lowers the volume of sugar solution required to reduce a given volume of Fehling's solution, the effect varying with the relative amounts of sucrose and the reducing sugar and with the titer. These corrections for the method employing 10 cc. of Fehling's solution are given in Table 33.

Notes.—In most cases the standardization may be omitted, the error generally being less than 1 per cent of the final result, provided the details of the analysis are carefully followed. Jackson[3] has shown that a variation of several minutes in the time of titration has very little influence on the result.

2. Gravimetric Methods.—The gravimetric methods, not requiring the preparation and standardization of an exact copper solution, are perhaps to be preferred for occasional rather than routine determinations of reducing sugars. A great number of gravimetric processes depending upon the use of Fehling's solution have been proposed, nearly all the older ones being limited

[1] *J. Soc. Chem. Ind.*, **1927**, 434T.

[2] *J. Assoc. Off. Agr. Chem.*, **1932**, 624.

[3] *J. Assoc. Off. Agr. Chem.*, **1930**, 201.

to the determination of a single sugar. For general use in food analysis, however, one of the later unified methods for the common reducing sugars is preferable. Of these, the method of Munson and Walker[1] is probably the one used most. While others are more rapid and convenient there are but few that approach it in precision. The comprehensiveness of the tables and the fact that the personal equation is not an important factor have added to its popularity. A compilation of the more important of the other methods, with their appropriate tables, may be found in Browne's "Handbook of Sugar Analysis."

TABLE 33*a*.—DEXTROSE IN THE PRESENCE OF SUCROSE
(Corrections in cc. to be added to burette readings.)

Burette readings, cc.	Sucrose/dextrose ratios			
	2/1	4/1	8/1	20/1
15	0.20	0.40	0.65	1.15
20	0.20	0.45	0.65	1.15
25	0.25	0.45	0.70	1.25
30	0.30	0.50	0.75	1.45
35	0.35	0.55	0.90	1.75
40	0.40	0.60	1.10	2.15
45	0.45	0.65	1.35	2.60
50	0.55	0.70	1.60	3.15

TABLE 33*b*.—LACTOSE IN THE PRESENCE OF SUCROSE
(Corrections in cc. to be added to burette readings.)

Burette readings, cc.	Sucrose/lactose ratios					
	3/1	6/1	10/1	12/1	15/1	20/1
15	0.15	0.30	0.60	0.75	0.90	1.10
20	0.25	0.50	0.80	0.95	1.15	1.45
25	0.30	0.60	0.95	1.15	1.40	1.75
30	0.35	0.70	1.10	1.30	1.55	2.00
35	0.40	0.80	1.20	1.45	1.70	2.05
40	0.45	0.90	1.30	1.55	1.75	2.10
45	0.50	0.95	1.40	1.60	1.80	2.15
50	0.55	1.05	1.45	1.65	1.85	2.20

Munson and Walker Method. *Preparation of Reagent. a. Copper Sulphate.*—Dissolve 34.639 grams of clear crystals of pure

[1] *J. Am. Chem. Soc.,* **1906,** 663; **1907,** 541; **1912,** 202.

copper sulphate, $CuSO_4 \cdot 5H_2O$, free from efflorescence, in water, add 0.5 cc. of strong sulphuric acid, and make up to 500 cc.

b. Alkaline Tartrate.—Dissolve 172 grams of the purest Rochelle salts (sodium potassium tartrate) and 50 grams of sodium hydroxide, free from carbonate, in water and make up to 500 cc. Allow it to stand 2 days and filter through glass wool or prepared asbestos.

Preparation of Asbestos.—First digest the asbestos, which should be of the amphibole variety, with hydrochloric acid (1:3) for 2 or 3 days. Wash free from acid and digest for the same length of time with sodium hydroxide solution (100 grams per liter), after which treat for several hours with hot alkaline copper tartrate (Fehling's solution). Then wash the asbestos free from alkali, digest it finally with nitric acid (sp. gr. 1.07) for several hours, wash free from acid, and keep it in water for use (see Notes).

Use the asbestos in porcelain Gooch crucibles, having a layer about ¼ in. thick, and wash it thoroughly to remove fine particles of asbestos, avoiding the use of too strong suction. Fill the crucible once with alcohol, once with ether, dry for 30 minutes at 100°C., cool in a desiccator, and weigh. Do not remove the asbestos after a determination but dissolve the cuprous oxide in hot dilute nitric acid and use the same felts over again, as they improve with use.

Determination.—Transfer 25 cc. each of the copper and alkaline tartrate solutions to a 400-cc. Jena or Pyrex beaker, and add 50 cc. of the reducing sugar solution, or, if a smaller volume of the sugar solution be used, add enough water to make the total volume 100 cc.

Heat the beaker upon an asbestos gauze over a Bunsen burner, so regulating the flame that boiling begins in 4 minutes, and continue the boiling for *exactly* 2 minutes, keeping the height of the flame the same as in the preliminary boiling. Keep the beaker covered with a watch glass during the entire time of heating. Without diluting, filter the cuprous oxide at once on an asbestos felt prepared as described above, using suction and a porcelain Gooch crucible. Wash the cuprous oxide thoroughly with water at a temperature of about 60°C., pour the hot solution out of the filter bottle, add 10 cc. of alcohol, and finally 10 cc. of ether. Dry for 30 minutes at 100°C., cool in a desiccator,

and weigh as cuprous oxide. Find the milligrams of reducing sugar corresponding to the weight of cuprous oxide from the appropriate column in Table 34, page 271.

Notes.—Filtration of boiling alkali is severe treatment for the asbestos mat. Munson and Walker meet this by very long digestion alternately with acid and alkali. There is often a strong temptation to curtail this long treatment, and this causes difficulty in the method. Brewster and Phelps[1] describe a shorter and equally satisfactory method of preparing the asbestos. Twenty-five grams of asbestos is boiled for 30 minutes with 250 cc. of 25 per cent sodium hydroxide (technical grade will be satisfactory; use a nickel or iron dish, or a Pyrex flask), then washed thoroughly, by suction, with boiling water. Most of the water is pressed from the asbestos and it is digested with 250 cc. of concentrated hydrochloric acid and 25 cc. of concentrated nitric acid for 30 minutes on the steam bath. Finally it is diluted with 250 cc. of hot water, filtered by suction and washed again thoroughly with boiling water. The process can be completed in 2 hours, in contrast with the long period of digestion prescribed by Munson and Walker.

The reagents should be the purest obtainable and the solutions, if turbid, should be filtered through glass wool until perfectly clear. Much of the trouble experienced in Fehling tests can be ascribed to impure chemicals. The solution and the asbestos should be tested by making "blank" determinations according to the regular procedure, except that no sugar is present. The crucible should not lose or gain in weight in a blank determination by more than 0.3 mg. The copper reduced by the reagents is due to the oxidizing effect of cupric copper on the alkaline tartrate. It varies with the copper concentration and would not be so great if a large proportion of the copper were rapidly reduced by the sugar, as is the case in an actual determination. Hence it is more common, after having determined that the asbestos is satisfactory within the limit stated, to use the table without applying a blank correction.

Close adherence to the details of the procedure as regards the volume of solution and time of heating is essential. The reduction of the copper solution is not complete, more copper being reduced if the solution is more concentrated or the time of heat-

[1] *Ind. Eng. Chem., Anal. Ed.*, **1930**, 373.

ing prolonged. Hence the given volume must be used, the solution boiled for exactly 2 minutes, making 6 minutes for the total time of heating, and the cuprous oxide filtered as soon as possible. In carrying out the method for the first time, it is advisable to regulate the flame for the 4-minute heating by a preliminary trial with 100 cc. of water.

The volume of sugar solution used in a determination should not contain, in general, more than 0.220 gram of a monosaccharide, 0.300 gram of lactose or 0.350 gram of maltose. Likewise, the lower ranges of concentration are involved in greater uncertainty than the ranges above concentrations of 50 mg. Autoreduction of the mixed copper reagent and slight changes in the weight of the asbestos may become quite large percentages of the reduced copper.

If very small amounts only of reducing sugar are known to be present a preferable method is that of Scales,[1] a volumetric method employing a copper solution (Benedict's) made with a milder alkali than Fehling's, which is at its best with the concentrations of sugar at which the usual methods are carried out with the greatest uncertainty.

In many food products, the reducing sugar determination is complicated by the presence of sucrose. Although sucrose does not reduce Fehling's solution directly, it is hydrolyzed to some extent by the hot Fehling's solution and thus gives small amounts of reducing sugar. The extent of this reducing action of sucrose is dependent upon the concentration of the sucrose and upon the amount of unreduced copper. Consequently, in accurate determinations of reducing sugar, it may be necessary to take the reducing action of the sucrose into consideration. The error may be avoided by taking an amount of the sugar solution such that the invert sugar will reduce nearly all of the copper, under which conditions the inversion of the sucrose is so slight as to be negligible. Such a method, suitable for less than 1.5 per cent of invert sugar with 97 per cent or more of sucrose, is that of Herzfeld,[2] largely used in sugar refineries. The Lane and Eynon method is also suitable. Another method, and the one adopted by Munson and Walker, is to determine and tabulate the copper

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[1] *Ind. Eng. Chem.*, **1919**, 747; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 478; *J. Assoc. Off. Agr. Chem.*, **1938**, 636.

[2] *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 481.

reduced by invert sugar in the presence of varying amounts of sucrose, separate columns being given in Table 34, page 271, for 0.4 gram and for 2 grams total weight of sucrose and invert sugar. It is generally possible from the other determinations made, as solids, ash, and polarization, to calculate how much of the material should be employed in the determination of reducing sugar in order that the total sugar shall be approximately one of the values noted. In other columns of the table are given the copper values for mixtures of sucrose and lactose, the proportions chosen being such as to be of value in the analysis of condensed milk and of milk chocolate, respectively.

The use of a table for calculating the amount of reducing sugar corresponding to a given amount of cuprous oxide is necessary on account of the variation in reducing power of the sugars with the concentration. In general, the reducing power of the monosaccharides decreases with increased concentration, and the same is true to a less extent of the disaccharides. The latter, however, are more subject to variations due to slight changes in the conditions attending the reduction, so that a definite law cannot be so well established for them.

The method of weighing the reduced copper as cuprous oxide, while convenient and accurate with sugars of reasonable purity, is not exact when working with impure solutions such as molasses and sirups. With these, organic or mineral impurities may be carried down with the precipitated copper, or a part of the copper may be precipitated in organic combinations rather than as the oxide. This condition can usually be determined by inspection of the cuprous oxide precipitate in the crucible. If it is not of the characteristic bright red color, but appears dull brown or various shades of yellow or greenish red, the result would better be checked by igniting to cupric oxide or by dissolving the precipitate and determining its content of copper.

Results of somewhat greater accuracy may be obtained by igniting the precipitate of cuprous oxide and weighing it as cupric oxide. This may be done by heating the crucible in a muffle at a red heat for 15 minutes or more simply by placing the crucible in a slightly larger one of platinum or nickel, and heating the outer crucible to bright redness for the same length of time. The crucible is then transferred to a desiccator and weighed when cool, bearing in mind the somewhat hygroscopic

TABLE 34.—MUNSON AND WALKER'S TABLE* FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES), AND MALTOSE (CRYSTALLIZED)

Cuprous oxide (CuO), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (CuO), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
10	8.9	4.0	4.5	1.6	......	6.3	6.1	......	6.2	10
15	13.3	6.2	6.7	3.9	......	9.4	9.1	......	10.4	15
20	13.8	8.3	8.9	6.1	......	12.5	12.1	......	14.6	20
25	22.2	10.5	11.2	8.4	......	15.7	15.2	......	18.7	25
30	26.6	12.6	13.4	10.7	4.3	18.8	18.2	......	22.9	30
35	31.1	14.8	15.6	12.9	6.5	22.1	21.3	......	27.1	35
40	35.5	16.9	17.8	13.2	3.8	25.5	24.7	......	31.3	40
45	40.0	19.1	20.1	17.5	11.1	28.9	28.0	......	35.4	45
50	44.4	21.3	22.3	19.7	13.4	32.3	31.3	......	39.6	50
55	48.9	23.5	24.6	22.0	15.7	35.8	34.6	......	43.8	55
60	53.3	25.6	26.8	24.3	18.0	39.2	37.9	......	48.0	60
65	57.7	27.8	29.1	26.6	20.3	42.6	41.3	......	52.1	65
70	62.2	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
75	66.6	32.2	33.6	31.2	24.9	49.4	47.9	44.8	60.5	75
80	71.1	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
85	75.5	36.7	38.2	35.8	29.6	56.3	54.6	50.7	68.8	85
90	79.9	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
95	84.4	41.1	42.7	40.5	34.2	63.2	61.3	56.6	77.2	95
100	88.8	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
105	93.3	45.5	47.3	45.2	38.9	70.0	68.0	62.6	85.5	105
110	97.7	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
115	102.2	50.0	51.9	49.8	43.6	76.9	74.6	68.5	93.9	115
120	106.6	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
125	111.0	54.5	56.6	54.5	48.3	83.8	81.3	74.5	102.2	125
130	115.5	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
135	119.9	59.0	61.2	59.3	53.1	90.6	88.1	80.5	110.5	135
140	124.4	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
145	128.8	63.6	65.9	64.0	57.8	97.5	94.8	86.5	118.9	145
150	133.2	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
155	137.7	68.2	70.6	68.8	62.6	104.4	101.5	92.6	127.2	155

*Condensed form. More extended tables may be found in BROWNE: "Handbook of Sugar Analysis," p. 66 (Appendix), or in *Assoc. Off. Agr. Chem.*, "Official Methods," 1935,

TABLE 34.—MUNSON AND WALKER'S TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRAM AND 2 GRAMS, TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES), AND MALTOSE (CRYSTALLIZED).— *(Continued)*

Cuprous oxide (CuO), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (CuO), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
160	142.1	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
165	146.6	72.8	75.3	73.6	67.4	111.3	108.2	98.6	135.5	165
170	151.0	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
175	155.5	77.4	80.1	78.4	72.2	118.2	114.9	104.7	143.9	175
180	159.9	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
185	164.3	82.0	84.9	83.2	77.1	125.1	121.7	110.7	152.2	185
190	168.8	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
195	173.2	86.7	89.6	88.0	81.9	132.0	128.5	116.8	160.5	195
200	177.7	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
205	182.1	91.4	94.5	92.9	86.8	138.9	135.3	122.9	168.9	205
210	186.5	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
215	191.0	96.1	99.3	97.8	91.7	145.8	142.0	129.0	177.2	215
220	195.4	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
225	199.9	100.8	104.2	102.7	96.6	152.7	148.8	135.2	185.5	225
230	204.3	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
235	208.7	105.6	109.1	107.7	101.6	159.6	155.6	141.3	193.8	235
240	213.2	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
245	217.6	110.4	114.0	112.6	106.5	166.6	162.4	147.5	202.2	245
250	222.1	112.8	116.4	115.1	109.1	170.1	165.8	150.6	206.3	250
255	226.5	115.2	118.9	117.6	111.5	173.5	169.2	153.7	210.5	255
260	231.0	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
265	235.4	120.0	123.9	122.6	116.5	180.5	176.0	159.9	218.8	265
270	239.8	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
275	244.3	124.9	128.9	127.7	121.6	187.4	182.9	166.1	227.1	275
280	248.7	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
285	253.2	129.8	133.9	132.7	126.6	194.4	189.7	172.4	235.5	285
290	257.6	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
295	262.0	134.7	138.9	137.8	131.7	201.3	196.5	178.7	243.8	295
300	266.5	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
305	270.9	139.7	144.0	142.9	136.8	208.3	203.3	185.0	252.1	305
310	275.4	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
315	279.8	144.7	149.1	148.1	141.9	215.3	210.2	191.3	260.4	315
320	284.2	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
325	288.7	149.7	154.3	153.2	147.1	222.2	217.0	197.6	268.7	325

TABLE 34.—Munson and Walker's Table for Calculating Dextrose, Invert Sugar Alone, Invert Sugar in the Presence of Sucrose (0.4 Gram and 2 Grams Total Sugar), Lactose, Lactose and Sucrose (2 Mixtures), and Maltose (Crystallized).—

(Concluded)

Cuprous oxide (CuO), mgm.	Copper (Cu), mgm.	Dextrose, mgm.	Invert sugar, mgm.	Invert sugar and sucrose		Lactose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Lactose and sucrose		Maltose, $C_{12}H_{22}O_{11}H_2O$, mgm.	Cuprous oxide (CuO), mgm.
				0.4 gm. total sugar, mgm.	2 gm. total sugar, mgm.		1 lactose, 4 sucrose, mgm.	1 lactose, 12 sucrose, mgm.		
330	293.1	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
335	297.6	154.7	159.4	158.4	152.3	229.2	223.9	204.0	277.0	335
340	302.0	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
345	306.5	159.8	164.6	163.7	157.5	236.2	230.8	210.3	285.4	345
350	310.9	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
355	315.3	164.9	169.8	168.9	162.7	243.2	237.7	216.7	293.7	355
360	319.8	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
365	324.2	170.1	175.1	174.2	167.9	250.2	244.6	223.1	302.0	365
370	328.7	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
375	333.1	175.3	180.4	179.5	173.2	257.2	251.5	229.6	310.3	375
380	337.5	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
385	342.0	180.5	185.7	184.8	178.5	264.2	258.5	236.0	318.6	385
390	346.4	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
395	350.9	185.7	191.0	190.2	183.9	271.2	265.4	242.5	326.9	395
400	355.3	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
405	359.7	191.0	196.4	195.6	189.2	278.2	272.3	249.0	335.2	405
410	364.2	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
415	368.6	196.3	201.8	201.0	194.6	285.3	279.3	255.5	343.5	415
420	373.1	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
425	377.5	201.7	207.3	206.5	200.0	292.3	286.3	262.1	351.8	425
430	382.0	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
435	386.4	207.1	212.8	212.0	205.5	299.3	293.3	268.7	360.1	435
440	390.8	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
445	395.3	212.5	218.3	217.5	211.0	306.3	300.3	275.3	368.4	445
450	399.7	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
455	404.2	218.0	223.9	223.0	216.5	313.4	307.3	281.9	376.7	455
460	408.6	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
465	413.0	223.5	229.5	228.6	222.0	320.4	314.3	288.5	385.0	465
470	417.5	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
475	421.9	229.0	235.1	234.2	227.6	327.5	321.2	295.2	393.3	475
480	426.4	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
485	430.8	234.6	240.8	239.9	233.2	334.5	328.2	301.8	401.6	485
490	435.3	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

nature of cuprous oxide. To convert cupric oxide into cuprous oxide, multiply by the factor 0.8994. It is of course necessary that the crucible and asbestos should have been previously ignited and weighed under similar conditions.

The only really satisfactory method when working with very impure products is to dissolve the precipitate of cuprous oxide and determine the copper volumetrically or by electrolysis. The volumetric method most commonly employed is that depending upon the liberation of iodine from potassium iodide, the reaction for cupric acetate being:

$$2Cu(C_2H_3O_2)_2 + 4KI \rightarrow Cu_2I_2 + 4KC_2H_3O_2 + I_2.$$

The procedure proposed by Low[1] has been adopted by the Association of Official Agricultural Chemists as a provisional method[2] but the modification suggested by Peters[3] is more rapid and convenient.

Peters' Modified Volumetric Iodide Method. *Procedure.*— Dissolve the cuprous oxide in the crucible in 5 to 10 cc. of concentrated nitric acid to which one-half its volume of water has been added. This is best done by covering the crucible with a small watch glass and allowing the acid to run beneath it from a pipette. After solution wash the crucible with 25 cc. of water, added in small portions. Rinse the solution into a 250-cc. Erlenmeyer flask, keeping the volume as small as possible; add 1 gram of pure powdered talc, and boil vigorously for 5 to 10 minutes. Cool to room temperature, make slightly ammoniacal and then slightly acid with acetic acid. Add 10 cc. of a saturated potassium iodide solution and titrate with $0.1N$ sodium thiosulphate until the brownish tinge has become faint, then add a few cubic centimeters of starch solution and continue the titration until the blue color due to iodine disappears. Toward the end of the titration, the blue color changes to a faint lilac, and the addition of the thiosulphate should be made rather slowly, drop by drop, until the solution clears. One or two trials will ensure a satisfactory end point.

Notes.—The chief objection to the iodide method, as formerly carried out, was in the danger of incomplete removal of the

[1] *J. Am. Chem. Soc.*, **1902**, 1082.
[2] *Assoc. Off. Agr. Chem.*, "Official Methods of Analysis," **1935**, p. 480.
[3] *J. Am. Chem. Soc.*, **1912**, 422.

nitrous acid formed in the solution of the cuprous oxide. The vigorous boiling and many minute bubbles caused by the addition of the finely powdered talc serve to remove the nitrous acid completely.

The thiosulphate solution employed should be standardized against a solution of pure copper in exactly the same manner as in a regular determination.

Electrolytic Method.—For occasional determinations of the reduced copper, or in cases where the longer time required is not objectionable, the electrolytic method is convenient and exact.

Transfer the asbestos and the cuprous oxide by means of a glass rod to a beaker and rinse the crucible with about 30 cc. of a boiling mixture of dilute sulphuric and nitric acids, containing 65 cc. of sulphuric acid (sp. gr. 1.84) and 50 cc. of nitric acid (sp. gr. 1.42) per liter. Heat and shake until solution is complete, filter, and wash. Return the asbestos to the crucible to be used for subsequent determinations. Electrolyze the solution, conveniently overnight, using a current density of about 0.25 ampere, or as described under the electrolytic determination of copper in any standard textbook on analytical chemistry.

Determination of Sucrose by Fehling's Solution.—Although in the majority of cases sucrose is most quickly and conveniently determined by the polariscope, in the absence of such an instrument, it may be determined with equal accuracy by means of Fehling's solution. As a matter of fact, in some foods containing only a small percentage of sucrose and a comparatively large amount of reducing sugars, as in honey, the determination by means of copper reduction is more exact than the optical method.

Since sucrose has no reducing power, it is, of course, necessary first to convert it to reducing sugar. This may be done by hydrolyzing it with dilute hydrochloric acid, 95 parts of sucrose yielding 100 parts of invert sugar.

This is the ratio of the molecular weights,

$$\underset{\text{Sucrose}}{\overset{(342)}{C_{12}H_{22}O_{11}}} + \underset{}{\overset{(18)}{H_2O}} \rightarrow \underset{\text{Dextrose}}{\overset{(180)}{C_6H_{12}O_6}} + \underset{\text{Levulose}}{\overset{(180)}{C_6H_{12}O_6}}; \quad {}^{342}\!/\!_{360} = 0.95$$

If then the resulting invert sugar be determined by Fehling's solution, 95 per cent of the weight of invert sugar will be the

corresponding weight of sucrose. If reducing sugars are present as well as sucrose, as is commonly the case in foods, the sucrose must be determined from the difference in reducing power before and after inversion. The method employed and the calculation can be best illustrated by an example.

Suppose that it is desired to determine sucrose in a raspberry sirup containing sucrose and invert sugar. Five grams of sirup are dissolved and made up to 500 cc. (Solution A). Another 5-gram portion is weighed, dissolved in about 75 cc. of water and inverted as on page 293, b. The inverted solution, after cooling, is nearly neutralized with sodium hydroxide, care being taken to leave it faintly acid, and made up also to 500 cc. (Solution B). In an aliquot part of each solution the reducing sugar is determined by the Munson and Walker method, page 267, calculating the results in each case as invert sugar.

Example.

		Mg. copper		Mg. invert sugar
50 cc. Solution B	=	407.7	=	226.1
50 cc. Solution A	=	195.4	=	101.7
				124.4

124.4 × 0.95 = 118.2 mg. = 23.64 per cent sucrose.
101.7 mg. = 20.34 per cent invert sugar.

If in any particular case the reducing sugar present should be some other sugar than invert sugar, it is still necessary in this determination to calculate it as invert sugar, in order to subtract the amount from the total to find the inverted sucrose.

Care should be taken to employ such an aliquot part for the final reduction that the amount of invert sugar present shall not exceed 240 mg., which is practically the limit of the Munson and Walker method. It may be necessary to calculate the correct amount to use from a preliminary determination.

OPTICAL METHODS

An important class of methods for determining the carbohydrates is based on their behavior toward polarized light. All the natural carbohydrates, as is true in general of organic substances possessing one or more asymmetric carbon atoms, possess the power of rotating the vibration plane of polarized light about an axis parallel to its direction of propagation. An asymmetric

carbon atom is one to which are attached four different atoms or groups of atoms. Rotations clockwise, looking toward the light source, are spoken of as *dextro*rotatory (+) and counterclockwise as *levo*rotatory (−). With substances in solution the angle through which this rotation occurs is in general directly proportional to the rotating power of the substance, the length of the column of solution traversed by the light and the concentration. Under definite conditions, then, the rotation produced may be used as a measure of the concentration.

Specific Rotation.—The unit in measurements of optical rotation is the specific rotating power of the substance or its *specific rotation*. This may be defined as the rotation in angular degrees of the plane of polarized monochromatic light which is produced by a solution of the optically active substance having a concentration of 1 gram in 1 cc. and polarized in a column 1 dm. long. This is given by the expression

$$[\alpha] = \frac{av}{lw},$$

where $[\alpha]$ is the specific rotation, a the degrees of angular rotation, l the length of the column of solution in decimeters, and w the grams of solute in v cc. of solution. If the concentration is expressed in percentage of substance in solution, as is sometimes the case, the formula is stated differently. Calling the percentage (grams of solute in 100 grams of solution) p and the specific gravity d, $w = pd$, and $v = 100$, hence

$$[\alpha] = \frac{100a}{lpd}.$$

The specific rotation depends further on the wave length of the light employed, the concentration and the temperature, and certain of these variables are customarily stated in expressing the value for any given substance. Thus the expression $[\alpha]_D^{20}$ means the specific rotation as determined for the D ray of the spectrum, more precisely 5892.5 angstrom units, and at 20°C., these being the usual standard conditions for measurement.

Effect of Temperature and Concentration upon Specific Rotation.—Variations in the concentration of the solution and in the temperature at which the observation is made have with many sugars a pronounced effect on the specific rotation. In the pres-

ence of invert sugar, for example, accurate polariscopic determinations of the sugar are impossible unless made at known temperatures. In general, the sugars show a decrease in specific rotation with increased temperature, the change being especially marked with levulose and arabinose. Xylose, on the other hand, shows a slight increase, and dextrose remains apparently unchanged at temperatures between 0 and 100°C.

The table below, arranged from Browne's "Handbook of Sugar Analysis," gives the correction for concentration or for concentration and temperature of the more common sugars:

Sucrose[1] $[\alpha]_D^{20} = +66.435 + 0.00870c - 0.000235c^2$
$\qquad\qquad$ (c = 0 to 65 grams per 100 cc.)

Dextrose[2] $[\alpha]_D^{20} = +52.50 + 0.018796p + 0.00051683p^2$
$\qquad\qquad$ (p = 0 to 100 per cent)

Levulose[3] $[\alpha]_D^t = -[101.38 - 0.56t + 0.108\,(c - 10)]$

Invert sugar $[\alpha]_D^t = -(27.9 - 0.32t)$

Lactose[4] $[\alpha]_D^t = +52.53 - 0.07(t - 20)\,[t = 15\text{ to }25°\text{C.}]$
$\qquad\qquad$ ($+52.53$ = constant for c = 2.4 to 40)

Maltose $[\alpha]_D^t = 140.375 - 0.01837p - 0.095t.$

[1] LANDOLT: "Das optische Drehungsvermögen," **1898**, p. 420.
[2] TOLLENS: *Ber.*, **1884**, 2238.
[3] JUNGFLEISCH and GRIMBERT: *Compt. rend.*, **1888**, 390.
[4] SCHMOGER: *Ber.*, **1880**, 1922.
[5] MEISSL: *J. prakt. Chem.*, **1898**, 114.

As stated above, there is no temperature correction for dextrose, and the temperature and concentration corrections for sucrose are so slight as to be negligible in food analysis.

The following values for $[\alpha]_D^{20}$ of the carbohydrates commonly occurring in foods will be found sufficiently exact for laboratory calculations:

Arabinose	+104.5
Dextrose	+ 52.5
Levulose	− 92.5
Invert sugar	− 20.0
Lactose (hydrate)	+ 52.5
Galactose	+ 80.5
Maltose	+138.5
Sucrose	+ 66.5
Xylose	+ 19.0
Starch } Dextrin	about + 195 for purified material.

Mutarotation.—The reducing sugars when freshly dissolved, or when a concentrated solution is diluted, often give at first

different polarization values which gradually change and become constant after some hours. For example, dextrose when first dissolved may give a rotation value of 105.2 instead of the constant value 52.5. This phenomenon is explained by the existence of two differing arrangements of the atoms within the sugar molecule, one configuration giving a high-rotating and the other a low-rotating form of the sugar. When freshly dissolved one of these forms may predominate, but upon standing they gradually reach an equilibrium. The constant rotation form is thus a mixture of the two in equilibrium.[1]

The constant rotation can be obtained by letting the solution stand for some hours, or instantaneously by bringing to a boil or adding about 0.1 per cent of ammonia, the rate of change to equilibrium being greatly accelerated by heat or by acids and alkalies. The possibility of mutarotation should be borne in mind when polarizing such food substances as honey or commercial glucose preparations.

The Polariscope. *Fundamental Concepts.*—This brief discussion is necessarily limited to the optical devices found in modern instruments. Polarized light is readily obtained by passing ordinary light in certain directions through crystals other than those belonging to the cubic system. Two rays are ordinarily produced, both of which are plane polarized, vibrating in mutually perpendicular planes. For use in polarizing apparatus crystals of calcite, Iceland spar, are commonly chosen, because in this substance the velocities
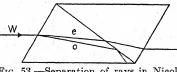
FIG. 53.—Separation of rays in Nicol prism.

of the two rays formed are quite different from each other; hence their paths in the crystal are quite divergent and they are more easily separated.

In order to eliminate one of the rays a thin layer of Canada balsam or similarly acting material is placed in the crystal in such a way that one ray only is transmitted, the device being the well-known Nicol prism, Fig. 53. The "ordinary" ray *o*, having for calcite a refractive index greater than that of the balsam, reaches it at more than the critical angle, is totally reflected and absorbed,

[1] For a full review and bibliography of the subject of Mutarotation, see HUDSON: *J. Am. Chem. Soc.*, **1910**, 889.

while the "extraordinary" ray *e* is transmitted, vibrating in the "principal section" of the crystal.[1]

When two Nicol prisms are so arranged that light passes successively through the two, if the principal sections of the two prisms are parallel, then all the light entering the second Nicol will be in its *extraordinary* ray vibration plane and will be entirely transmitted; if the principal section of the second Nicol is perpendicular to that of the first (the position of "crossed Nicols") then the entering light will be in the *ordinary* ray vibration plane of the second Nicol, hence will be totally reflected and absorbed, no light being transmitted; in intermediate positions of the Nicols the light transmitted by the system will be between "total
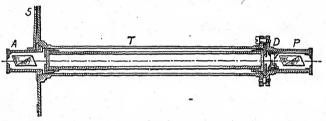
Fig. 54.—Essential parts of polariscope. (*Rolfe.*) *A*, revolving sleeve containing eyepiece and analyzer; *S*, scale showing position of rotation of analyzer; *T*, tube of optically active solution; *P*, polarizer; *D*, end-point device.

extinction" and "maximum illumination," becoming brighter as the Nicols approach the parallel position.

Theoretically, then, if two such prisms are placed in the position of crossed Nicols (total extinction) and between them is placed an optically active substance capable of rotating the vibration plane of the polarized ray, say 15° clockwise, the field will no longer be totally dark and the second prism would have to be rotated 15° clockwise to restore the dark appearance, thus measuring the amount of rotation. Actually, it is difficult for the eye to distinguish this total extinction end point, so that some additional optical device (end-point device) is used to replace the dark field by a divided field with equal intensities of light in the two portions.

Construction.—In Fig. 54 are shown the essential parts of a polariscope. The arrangement of the prisms is essentially the

[1] Recognizable in many forms of Nicol prism by being the short diagonal of the end faces.

same in all forms, monochromatic light being transmitted as polarized light by the *polarizer*, passing thence through a tube of definite length and provided with glass ends, containing the solution of the optically active substance, the amount of rotation being measured on a circular scale by rotating the second Nicol prism or *analyzer*. The main difference found in various instruments consists in the end-point device employed.

End-point Devices.—The function of the end-point device is to transmit to the analyzer, instead of a single ray from the polarizer, two rays, the vibration planes of which make a slight angle with each other and equal angles, a little less than 90°, with the
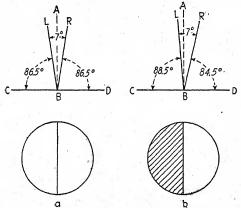
Fig. 55.—Illustrating effect of end-point device.

vibration plane of the analyzer, hence produce after passing the analyzer a divided field, faintly but evenly illuminated on the two sides, in fact a *balanced* field.

Figure 55 will aid in making this clear. *AB* (in *a*) represents the original vibration plane of light from the polarizer, *CD* that of the analyzer (crossed Nicols), while *LB* and *RB* are the vibration planes that reach the analyzer on account of the end-point device, each illuminating one-half the field. Since the angles *LBC* and *RBD* are equal and nearly 90° (usually about 86.5°), the two halves of the field will be faintly and *evenly* illuminated. Note, however, that this effect is produced in one half of the field by a *right*, in the other half by a *left*, rotation. If now a tube of sugar solution, rotating say 2° clockwise, be placed between the end-point device and the analyzer the effect

will be as in *b.* The vibration plane of the rays on the left side of the field being nearer 90° to that of the analyzer, the left side of the field will have less illumination than the right, *i.e.*, a shadow will be produced in that half of the field. Equal intensity of illumination on the two sides can be restored only by rotating the analyzer clockwise 2° until the angles made with the analyzer vibration plane are again equal. Three such end-point devices are commonly used on modern instruments.

a. The Jellet-Cornu or *"split" prism,* which consists of an ordinary Nicol prism that has been bisected along its principal section and small wedge-shaped sections removed, after which the prism is cemented together again. The effect of this is to incline slightly toward each other the planes of polarization of the light illuminating the two halves of the field. The split prism being used as a polarizer, the field produced by rotating the analyzer until the Nicols are "crossed" will not be black, as would be the case with ordinary Nicol prisms, but shows a faint uniformly lighted field or zero point. The slightest rotation of the analyzer from this position causes a shading in one-half of the field as explained above.

b. The Lippich Polarizer.—The shadow device proposed by Lippich consists of a small Nicol prism mounted in front of the polarizer so as to cover one-half the field. By rotating the small Nicol slightly from a parallel position with the polarizer (remember that the light leaving the Nicol prism still vibrates in its principal section, *i.e.*, the vibration plane of the transmitted ray rotates with the Nicol) two vibration planes making a small angle with each other can be set up as with the "split prism," with the added advantage that the angle can be varied to suit conditions. It is probably the most sensitive of the end-point devices in common use but, being unsymmetrical, it has the slight disadvantage that variations in the half-shadow angle produce changes in the zero point of the instrument. By similarly using two small Nicols the field may be divided into three bands, so-called "triple field" polariscopes, which are somewhat more sensitive.

c. Laurent End-point Device.—Less commonly used than the two forms just described is the "half-wave plate" of Laurent, which has the advantage of variable sensitivity without altering the zero setting, but the disadvantage that its use is restricted to

monochromatic light and commonly to the sodium ray. It consists of a thin plate of quartz cut parallel to its optic axis. Such a section of quartz is doubly refracting and the thickness is such that one component of the light has its vibrations retarded half a wave length. The effect of this is the same as with the Jellet-Cornu prism, giving two planes of vibration which are inclined toward each other in each half of the field by a small angle, but with the decided advantage that this angle can be varied by means of the rocking polarizer.
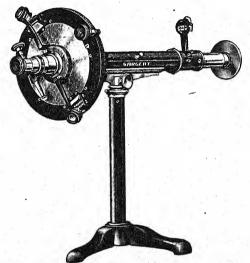
Fɪɢ. 56.—Rotary polariscope with Lippich polarizer.

Figure 56 shows a typical rotary polariscope or polarimeter. Note the small pointer and scale mounted above the polarizer, which is used in connection with the variable half-shadow angle of the Lippich half Nicol. Figure 57 illustrates a modern sodium-vapor arc lamp which gives a steady intense light many times stronger than the salt burners ordinarily used.

It should be borne in mind that the rotary polariscope can be used only with monochromatic light. This is in no way due to the construction of the instrument but to the fact that the sugar or other optically active substance rotates the vibration plane of polarized light differing degrees for the different wave lengths, the red being rotated least and the violet most. The field at the end point, then, will be colored, different colors being

observed for different concentrations of sugar, and at other positions the two halves of the field will exhibit contrasting colors.

The Saccharimeter.—Logical as the angular degree scale of the polarimeter may be for reading rotations, it is not a convenient scale for sugar determinations. The amount of sucrose in 100 cc. of solution which would read directly in per cent, *i.e.*, read 100°
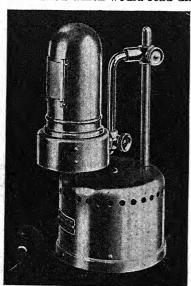
for the pure sugar, would be about 75 grams for the angular degree scale, and other common sugars, as dextrose and lactose, would require even more. Further, the light sources most common in the laboratory for monochromatic light are comparatively feeble. For these reasons there has always been a commercial demand for a special polariscope for sugar work that will use white light and be based on a smaller amount of sugar. Such an instrument is found in the quartz-wedge *saccharimeter*. In this the polarizer and analyzer are stationary and the rotation caused by the sugar solution is measured by interposing a wedge of optically active quartz until the rotation of the sugar is exactly compensated by that of the quartz. Readings are made on a scale attached to the quartz wedge.

White light may be used in place of monochromatic since the dispersive power of quartz and of sugar in water solution is very nearly the same; and hence if the rotation of the sugar is compensated by the oppositely rotating quartz, it follows that the rotational *dispersion* of the sugar will also be neutralized (compensated) by the opposite dispersion of the quartz. The correction of dispersion is not absolute, the blue and violet wave lengths causing some trouble, so that in precise work a light filter of some kind should be used (page 288). This means further that the

quartz compensation saccharimeter can be used only with substances resembling sucrose in rotational dispersion, unless monochromatic light be employed. Even then the range of the instrument is limited, its maximum value corresponding to only about 35 angular degrees. It is primarily for sugar solutions only.

The arrangement of the optical parts in the simpler form of quartz-wedge compensator, a single-wedge system, will be clear from the diagram (Fig. 58). AB represents the line of vision, the eye of the observer being at A. C and D are two wedges of dextrorotatory quartz, of which C is movable and D not. E is a fixed section of levorotatory quartz. The two wedges together make a section of parallel sides to E, the thickness of which can be varied by moving C. At the zero point the combined thicknesses of C and D are equal to E. If, however, a tube of dextrorotatory sugar solution be placed in the instrument between the polarizer and the compensation plate E, it will be necessary to restore the
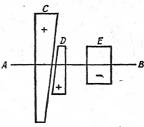
Fig. 58.—Diagram of quartz-wedge compensator. (*Rolfe.*)

optical neutrality by moving C until sufficient less dextrorotatory quartz is interposed to compensate for the dextrorotation of the sugar. If the sugar solution should be levorotatory it will of course be necessary to move C in the opposite direction. The quartz wedges can be equally well *levorotatory*, in which case the compensation plate E must be dextrorotatory.

A more complicated form is the double-wedge system, in which the compensation plate is replaced by another pair of wedges of opposite rotation. This has the advantage that any reading obtained by the working wedge can be verified by a second reading taken on the control wedge.

A general idea of the appearance of the saccharimeter may be gained from Fig. 59, which shows a standard type of instrument in use in many laboratories, and the arrangement of the parts is well shown in Fig. 60, which has reference in this case to a double-wedge compensator.

Beginning at the left of the figure, which is the end of the instrument nearest the observer, 1 and 2 are the eyepiece and

objective, respectively, of the telescope through which the field is viewed; 3 is the Nicol prism or analyzer; the two pairs of quartz wedges, 4, 5, and 6, 7, constitute the double-wedge compensator; 8 is a lens; 9 is the second Nicol prism or polarizer, the tube of solution being inserted between 8 and 9; 10 and 11, at the end nearest the source of light, are the collimating lenses.
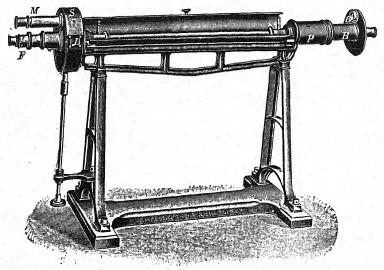
FIG. 59.—Quartz-wedge saccharimeter.

The end-point device in the saccharimeter is the same as in the polariscopes previously mentioned, the Jellet-Cornu prism and the Lippich polarizer being the forms employed. The Laurent, being only for monochromatic light, is obviously

FIG. 60.—Diagram of saccharimeter with double-wedge compensator.

inapplicable. Any convenient source of illumination that furnishes a steady white light may be used.

For an extended discussion of the construction of polariscopes and saccharimeters, the student is referred to the following special treatises:

Browne: "Handbook of Sugar Analysis."
Landolt: "Optical Rotation of Organic Substances" (trans. by Long).
Rolfe: "The Polariscope in the Chemical Laboratory."

Scale and Normal Weight.—The scale of a saccharimeter is so graduated as to read 100° when a certain definite weight (the *normal weight*) of pure sucrose is dissolved in 100 cc. at a definite temperature and polarized in a 200-mm. tube at the same temperature. This standard was fixed originally by Ventzke as the rotation of a solution of sucrose having a specific gravity of 1.100 at 17.5°C., corresponding to 26.048 grams of sucrose in 100 cc.

Since then the saccharimeters in common use have been variously graduated for 26.048 grams of sucrose in 100 *Mohr* cubic centimeters; for 26.048 grams of sucrose in 100 *true* cubic centimeters;[1] and finally, following the recommendations of the International Sugar Commission (1900), for 26.000 grams of sucrose in 100 true or metric cubic centimeters, which is practically the same as the first. The value is almost the same for the milliliter.

Critical studies both in this country and abroad[2] with specially purified sucrose, however, have since shown that the International standard of 26 grams is slightly in error, the true reading being 99.90° instead of 100°; hence that 26.026 grams of sucrose should be the normal weight for saccharimeters previously standardized for 26 grams in accordance with the Herzfeld-Schönrock conversion factor of 0.34657 for the *D* line of sodium light.[3] It is therefore essential, more than ever, that the exact standard for the instrument should be known, errors from neglect of this precaution amounting to possibly several tenths of 1 per cent. If at all in doubt, the normal weight should be checked by means of a rotary polariscope, standard quartz plate (preferably

[1] The Mohr cubic centimeter is the volume occupied by 1 gram of water at 17.5°C., weighed in air with brass weights; the true or metric cubic centimeter is the volume of 1 gram of water at 4°C., weighed *in vacuo*. (1 Mohr cc. = 1.00234 true cc. = 1.00231 milliliters.)

[2] Stanek: *Z. Zuckerind. čechoslovak. Rep.*, **1921**, 417, 425; Kraisy and Traegel: *Z. Ver. deut. Zucker-Ind.*, **1924**, 193; Bates and Jackson: *U. S. Bur. Standards, Sci. Paper* **268**, (1926), Browne: *J. Assoc. Off. Agr. Chem.*, **1929**, 106.

[3] Bates and Jackson [*Bur. of Standards, Sci. Paper* **268**, (1926)] found the value 0.3462.

certified by the Bureau of Standards), or a carefully calibrated control tube.

The International Sugar Commission (1932) adopted an "International Sugar Scale"[1] (°S) setting the 100°S. = 34.620° (angular) ± 0.002 (λ = 5892.5 Å.) at 20°C. The reading of the normal sugar solution on the Herzfeld-Schönrock scale was accepted as 99.90°S. The values for the scales most commonly used are summarized:

1° Ventzke Sugar Scale (°V.)   = 0.34657° Angular Rotation *D*.

1° Angular Rotation *D*   = 2.88542° Ventzke Sugar Scale.

Normal weight Ventzke Sugar Scale = 26.026 grams.

1° International Scale (°S.)   = 0.34620° Angular Rotation *D*.

1° Angular Rotation *D*   = 2.88850° International Scale.

Normal weight International Scale   = 26.000 grams.

Other values for various scales will be found in the "Official Methods" of the Association of Official Agricultural Chemists, **1935,** p. 468. It should be noted, however, in using these values, that at the 1936 meeting of the Sugar Commission the French scale normal weight was changed from 16.29 grams to 16.269 grams.

Bichromate Light Filter.—When polarizing solutions of some sugars, even with pure sucrose at high rotation, the two halves of the saccharimeter field show slight differences of tint at the zero point. This is due to the fact that these solutions are not of exactly the same dispersive power as quartz, and it causes slight differences in readings with different observers. This error can be readily eliminated by passing the light through a cell of potassium bichromate solution, placed in the end of the saccharimeter nearest the light. The proper strength is a 3 per cent solution of potassium bichromate in a cell 3 cm. long. For polarizing solutions containing commercial glucose[2] a bichromate cell is necessary for accurate work, and it is advantageous to use

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[1] Also called Bureau of Standards Scale.

[2] Browne (U. S. Dept. Agr., *Bur. Chem. Bull.* **122,** p. 221) prefers for such material a solution of twice the above concentration.

it in all polarizations of food products. If the polariscope available is not provided with a cell for bichromate solution, an aurantia screen placed between the lamp and the instrument will be found a very fair substitute.[1]

Determination of Sucrose by the Polariscope. a. Direct Method.—If sucrose is the only sugar present, the following simple method will be adequate for its determination.

Preparation of the Solution.—Weigh out the normal weight of the sample, conveniently in a "sugar dish" (Fig. 61). Weigh quickly to avoid loss by evaporation and weigh only to the nearest 0.005 gram. It is of course permissible to take some other suitable weight and calculate the results to the normal weight. Transfer the weighed sample by means of a small amount of water to a properly calibrated 100-cc. flask. The total volume should not exceed 60 to 65 cc. Dissolve by shaking and, if the solu-

Fig. 61.—Sugar dish for weighing samples.

tion is not perfectly clear and nearly free from color, clarify it by adding 2 to 5 cc. of basic lead acetate or occasionally 6 to 10 cc. of alumina cream.[2] In any case it is desirable to use only as much clarifier as is absolutely necessary, since an excess may cause serious error, as explained on page 291. When the right amount of lead has been added, a flocculent precipitate usually settles, leaving a clear solution above. Make up to the 100-cc. mark

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[1] Such a screen may be easily made by taking a small photographic plate, fixing it in hyposulphite without exposure to light, washing and soaking it in an alcoholic solution of the dye Aurantia for 10 to 15 minutes. The plate is then rinsed and dried.

[2] These reagents are prepared according to the methods of the Association of Official Agricultural Chemists, "Official Methods of Analysis," **1935**, p. 468, as follows:

1. Basic Lead Acetate.—Prepare by boiling 430 grams of normal lead acetate, 130 grams of litharge, and 1,000 cc. of water for $\frac{1}{2}$ hour. Allow the mixture to cool and settle, and dilute the supernatant liquid to 1.25 sp. gr. with recently boiled water. Dry lead subacetate (560 grams) may be substituted for the normal salt and litharge, if desired.

2. Alumina Cream.—Prepare a cold saturated solution of alum in water. Add ammonium hydroxide with constant stirring until the solution is alkaline to litmus, allow the precipitate to settle and wash by decantation with water until the wash water gives only a slight test for sulphates with barium chloride solution. Pour off the excess water and store the residual cream in a stoppered bottle.

at 20°C. and mix thoroughly. If foam obscures the meniscus, a drop or two of ether will remove it. Filter the solution through a dry filter, rejecting the first 15 to 20 cc. of the filtrate, and keeping the funnel covered with a watch glass to avoid evaporation. Rinse the polariscope tube twice with small portions of the solution and fill it so that no air bubbles remain. See that the outside of the cover glasses is clean and dry and examine the solution by looking through the tube lengthwise before placing it in the instrument. If the solution is not perfectly clear and bright, it will be useless to try to read it. It is convenient to make all polarizations in a tube provided with an opening on the side through which a thermometer can be inserted to take the temperature, as shown in Fig. 62.
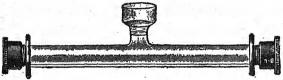
Fig. 62.—Polariscope tube with opening for thermometer.

Zero Error.—It is seldom that the instrument reads exactly zero, so any deviation must first be determined.

See that the instrument is pointed directly at the source of light, which should be about a tube length (200 mm.) from the end of the saccharimeter. Set the scale a few divisions from zero and focus the eyepiece so that the field and the dividing line are perfectly clear and sharp. Rotate the milled head until the field is uniformly illuminated. It is usually best to do this by moving it very slightly on either side of the zero point and observing when the shadow just flits across the dividing line. Take the average of five separate readings made in this way as the *zero error.*

Polarization of the Solution.—This is made at 20°C. in the manner just described for the zero reading, taking the average of five readings, and correcting for the zero error.

Notes.—In thus measuring a physical property readily affected by changes of concentration and temperature, certain precautions are absolutely necessary. Care should be taken to avoid loss by evaporation during weighing and filtration; changes of temperature by close proximity of heat to the instrument or by

careless handling of the observation tube must be carefully prevented.

By far the greater source of error, however, especially with the complex mixtures that may be met in foods, is in clarification. These errors are due mainly to the use of basic lead acetate in excessive amount and are (1) volume occupied by the lead precipitate, increasing the concentration of the solution; (2) precipitation of basic lead salts of dextrose and levulose; and (3) change in specific rotation of levulose due to the formation of a soluble dextrorotatory lead levulosate. The error due to volume of precipitate is ordinarily neglected in food work. If desired, however, it can be estimated and corrected by the method of double dilution,[1] or dry lead subacetate may be substituted for the solution generally used. (Horne's dry defecation method.[2]) The other two sources of error are obviously of less consequence if the sucrose alone is to be determined by double polarization (see below) as is customary in food analysis. In any case, however, only the smallest amount of basic lead acetate necessary to clarify the solution should ever be used. The quantities generally employed do not affect the specific rotation of sucrose.

b. Clerget's or Double Polarization Method.—In the presence of other optically active substances, the percentage of sucrose evidently cannot be determined by the simple polarization method just described. The polariscopic reading of a solution is the resultant rotation of all optically active substances present. In nearly all saccharine food products other sugars than sucrose, some dextro-, others levorotatory, are present; hence the first polariscopic reading is not a true measure of the sucrose but must be supplemented by a second, in which the rotation of the other sugars is kept constant, while that of sucrose is subjected to a change that can be measured and is known to be an exact function of the quantity of sucrose. This function is known as the "Clerget constant" or "divisor."

By hydrolysis or "inversion" the sucrose molecule is split, with the addition of 1 molecule of water, into 1 molecule of dextrose and 1 of levulose. It is upon the ease with which this split occurs that the classic method of Clerget depends. If D

[1] SCHEIBLER: *Z. Ver. deut. Zucker-Ind.*, **1875**, 1054; BROWNE: "Handbook of Sugar Analysis," p. 209.

[2] HORNE, W. D.: *J. Am. Chem. Soc.*, **1904**, 186; **1907**, 926.

represents the change in polariscopic reading before and after inversion and t the Centigrade temperature, the percentage of sucrose will be given by the formula

$$S = \frac{100D}{C - at},$$

in which C is the Clerget constant and a its temperature coefficient. C of course represents the change in rotation of pure sucrose before and after inversion.

A solution of the normal weight of sucrose in 100 cc., polarizing $+100°$ on the saccharimetric scale, will, when inverted by the method described on page 294, polarize $-43.0°$ at 0°C.

Since the levorotation of invert sugar decreases practically 0.5° for each degree increase in temperature because of the decrease in specific rotation of levulose, the reading at $t°$ would be $-[43.0° - (0.5t)]$.

At 20°C., for example, the reading would be $-33.0°$. Since the change in rotation has been from $+100°$ to $-[43.0° - 0.5t]$, or $143.0 - 0.5t$, this value may be used as a measure of the amount of sucrose present; provided, of course, that this change in rotation is due to the sucrose alone. Actually, the value varies slightly with concentration so that the expression becomes a little more complicated.

For analytical purposes the hydrolysis may be brought about by either of two catalytic agents, the enzyme invertase or hydrochloric acid. The first of these, from the standpoint of accuracy and the avoidance of disturbing side reactions, is the ideal hydrolyzing agent. It is perfectly selective in its action upon the biose sugars. Although too slow in its action for routine analyses, it is the method to follow when the highest accuracy is demanded. The invertase can be purchased, or directions for the preparation and use of a suitable solution will be found in the methods of the Association of Official Agricultural Chemists.[1]

The methods employing hydrochloric acid as the inverting agent are the ones most widely employed, the temperature of inversion ranging ordinarily from 20 to 70°C. In the case of food materials in general, where mixtures of sugars are frequently

[1] "Official Methods of Analysis," **1935**, 470: JACKSON and McDONALD: *J. Assoc. Off. Agr. Chem.*, **1939**, 582.

encountered, the inversion at low temperatures is decidedly to be chosen, and it is therefore given preference in the description below which follows quite closely the "Official Methods."

Procedure. *a. Inversion at Room Temperature.*—Dissolve double the normal weight of sample in water in a 200-cc. flask with the precautions detailed on page 289, clarify as there described, dilute to 200 cc., and filter, rejecting the first 25 cc. of the filtrate. Remove the lead from the filtrate by adding dry, powdered potassium oxalate, a little at a time, avoiding any excess, mix well, and filter again through a dry filter, rejecting the first 25 cc. of the filtrate. Pipette 50 cc. of the lead-free filtrate into a 100-cc. flask, make up to the mark, mix thoroughly and polarize in a 200-mm. tube, as described on page 290. The result, multiplied by 2, is the direct reading (*P* of the formula given below) or polarization before inversion.

To 50 cc. of the lead-free filtrate add 10 cc. of hydrochloric acid (sp. gr. 1.029 at $\frac{20°}{4°}$) and set aside for 24 hours at a temperature between 20 and 25°C.; or if the temperature is between 25 and 30°C., set aside for 10 hours. Make up to 100 cc., mix well, and polarize in a 200-mm. tube provided with a side opening and a thermometer. The polarization is preferably made at 20°C., but if it is necessary to work at any other temperature, which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at the same temperature. The result, multiplied by 2, is the invert polarization, or *I*.

Calculate sucrose by the following formula:

$$S = \frac{100(P - I)}{143.2 + 0.0676(m - 13) - t/2},$$ in which S = percentage of sucrose; P = direct reading, normal solution; I = invert reading, normal solution; t = temperature at which readings are made; and m = grams of total solids in 100 cc. of the invert solution read in the polariscope.

Determine total solids as percentage by weight, using the refractometer as directed on page 315, and multiply by the specific gravity as obtained from Table 36, page 309.

b. Inversion by Heating.—Prepare the solution of the sample as under *a*, using as there double the normal weight in a 200-cc. flask, this giving ample volume of the prepared solution for

further treatment. Depending upon the color or character of the product, fractions of the normal weight may be used and the results reduced by calculation to the basis of the normal weight per 100 cc.

Pipette one 50-cc. portion of the lead-free filtrate into a 100-cc. flask, dilute with water to the mark, mix well, and polarize in a 200-mm. tube. The result, multiplied by 2, is the direct reading (P of formula on page 293) or polarization before inversion.

For the invert reading, pipette another 50-cc. portion of the lead-free filtrate into a 100-cc. flask, and add 25 cc. of water. Then add, little by little, while rotating the flask, 10 cc. of hydrochloric acid (sp. gr. 1.029). Heat a water bath to 70°C. and regulate the burner so that the temperature of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates 67°C. This preliminary heating period should require from $2\frac{1}{2}$ to $2\frac{3}{4}$ minutes. From the moment the thermometer in the flask indicates 67°C., leave the flask in the bath for exactly 5 minutes longer, during which time the temperature should gradually rise to about 69.5°C. Plunge the flask at once into water at 20°C. When the contents have cooled to about 35°C., remove the thermometer from the flask, rinse it, and fill almost to the mark. Leave the flask in the bath at 20°C. for at least 30 minutes longer, and finally make up exactly to volume. Mix well and polarize the solution at 20°C. in a 200-mm. tube provided with a side opening and thermometer, as under *a*. This reading must also be multiplied by 2 to obtain the invert reading, I of the formula on page 293.

Calculate sucrose as under *a*, noting that in this case the value 143.2 of the formula should be 143.0. Note also that $P - I$ means the *algebraic difference*, which in actual figures may be the *sum* of the two readings.

Notes.—The methods as described should be carefully followed, especially as regards the time and temperature of inversion. Levulose is easily decomposed during inversion, and its optical rotation is greatly affected by slight changes of temperature.

The Clerget constant, as given on page 292, is correct only for a solution containing the inverted half-normal weight in 100 cc., which would be the case if the normal weight were taken originally; hence the correction for the concentration of the

solution used for invert polarization. Moreover, this correction must be for the concentration of total sugars, since the specific rotation of sugar mixtures is not determined by the specific rotation of the components at their partial concentration, but by that at the total sugar concentration.[1] It is important, also, to have the solutions for both direct and invert polarizations at the same concentration.

The value for the Clerget divisor is also affected by the rate of decomposition of invert sugar in hydrochloric acid at various temperatures, a certain amount of destruction always occurring, the strength of the hydrochloric acid, the influence of impurities, such as salts, upon the specific rotation of both sucrose and invert sugar, and other factors. A particularly careful study of the hydrochloric acid method for the inversion of sucrose has been made by Jackson and Gillis[2] to whose paper the student is referred. Reference should be made also to the criticisms of Browne[3] and of Hinton.[4]

It should be remembered in all of these considerations that factors worked out for pure sucrose solutions are not absolutely exact for the less pure food products.

The effect of the hydrochloric acid present during polarization on the specific rotation of levulose should also be borne in mind in some cases. In the polarization of jams or honey, for example, an error is caused by the presence of considerable quantities of invert sugar, which would have a different rotation in the direct polarization in neutral solution than in the invert polarization in the presence of hydrochloric acid.

An invert sugar concentration, for instance, which in aqueous solution reads -42.00, if read in the presence of hydrochloric acid as in the usual inversion process would read -43.25. This change would, in the usual course of analysis, be reported as sucrose. If much invert sugar, then, is present in the original sample, the direct polarization is best made in the presence of some substance that will cause a similar increase in the rotation

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[1] Vosburgh: *J. Am. Chem. Soc.*, **1921,** 219. Recent work indicates that the value of the concentration coefficient given may be too low, Jackson and McDonald (*J. Assoc. Off. Agr. Chem.*, **1939,** 582) finding 0.0794 instead of 0.0676.

[2] *U. S. Bur. of Standards, Sci. Paper* **375** (1920).

[3] *Intern. Sugar J.*, **1921,** 516.

[4] *Ibid.*, 689.

of invert sugar to that produced by hydrochloric acid. Such a substance, according to Jackson and Gillis,[1] is sodium chloride, and the method described on page 293, is readily modified by adding to the 50 cc. of the lead-free filtrate, before diluting to 100 cc. for direct polarization, 2.315 grams of sodium chloride. Carry out the determination in all other respects as described and calculate the sucrose by the same formula, noting, however, that the value 143.2 in the formula should in this case be substituted by 142.63.

If results of the greatest accuracy are not necessary, the double polarization method may be carried out somewhat more simply, without correcting for concentration, as follows:

Free the clarified solution used for direct polarization (page 289) from lead by cautiously adding successive small portions of potassium oxalate until no more lead is precipitated. Avoid an excess. Filter through a dry filter. Place 50 cc. of the lead-free filtrate in a 100-cc. flask, add 5 cc. of concentrated hydrochloric acid (sp. gr. 1.19) and allow the flask to stand at 20 to 25°C. (room temperature) for 20 to 24 hours, make up to 100 cc., and polarize in a tube provided with a thermometer by which the exact temperature at the time of polarizing may be noted. It is preferable, as in the direct polarization, to make the reading at 20°C.

Sucrose is calculated by the formula

$$S = \frac{100(P - I)}{143.0 - \dfrac{t}{2}},$$

in which the notation is the same as on page 293.

With food products in general, the double polarization method cannot be relied upon much closer than 0.5 per cent. With pure sugars, of course, the accuracy is much greater.

Calculation of Other Sugars from the Double Polarization.— If only one other sugar is present besides the sucrose, its amount may be calculated fairly closely by the formula:

$$X = \frac{66.5(P - S)}{sp},$$

where P is the direct polarization (for the normal weight), S

[1] *U. S. Bur. of Standards, Sci. Paper* **375**, Method IV, p. 187.

the percentage of sucrose as calculated from the double polarization, 66.5 the specific rotation at 20°C. of sucrose, and *sp* the specific rotation at 20°C. of the second sugar.[1]

Example.—A sample of jelly, in which sucrose and invert sugar are present, polarizes directly +47.0°, and after inversion polarizes −20.1° at 20°C. (page 296). What is the percentage of sucrose and of invert sugar?

$$\text{Sucrose} = \frac{100[47.0 - (-20.1)]}{143.0 - \dfrac{20}{2}} = 50.5 \text{ per cent.}$$

$$\text{Invert sugar} = \frac{66.5(47.0 - 50.5)}{-20} = 11.6 \text{ per cent.}$$

Note.—With most food products, the result obtained in this way is not strictly exact on account of the errors inherent in the sucrose determination, as well as the error caused by the occlusion of reducing sugars in the basic lead precipitate. With most sugars, also, the value taken for the specific rotation is only an average, not an exact value, varying commonly with concentration.

Polarization at 87°C.—Considerable use is made in food analysis of polarization at higher temperatures than 20°C., especially of polarization of the inverted solution at 87°C. in the approximate determination of commercial glucose.

Solutions of invert sugar become apparently optically inactive when heated to a temperature of about 87°C. This is on account of the decrease in specific rotation of levulose with increasing temperature until it just equals that of the dextrorotatory dextrose. The exact temperature of compensation varies somewhat with the concentration, but 87°C. is the value ordinarily used. If we take the case of a table sirup in which the sugars present are sucrose, invert sugar, and commercial glucose, by polarizing the inverted solution at 87° the invert sugar and inverted sucrose will read practically zero, leaving the commercial glucose to be determined directly from the polariscopic reading. Certain exceptions to this statement will be noted later.

The saccharimeter ordinarily employed may be used to make the readings at 87°C., but on account of the considerable difference in coefficient of expansion between glass and metal, and the

[1] See table on p. 278.

consequent difficulty of securing a tight joint with successive heating and cooling, an all-metal, preferably silver, jacketed tube is best for the solution.   Care should be taken that the inner tube is not of too small bore; otherwise the bubbles formed during the heating are likely to prove troublesome.   The hot water for the jacket may be supplied from a large tank of boiling water placed at a little distance from this instrument and high enough so that the hot water may flow by gravity through a rubber tube, the temperature being controlled by regulating the flow with a stopcock.   Sy[1] has devised a simple and practical plan for this purpose.   Great care should be taken to avoid heating the instrument.   It is better to heat the tube while outside of the trough and place it in position only when nearly at the desired temperature.

Both on account of employing a metal tube and to avoid any action of the acid on the commercial glucose during the heating, it is best to polarize a separate neutral solution as follows:

Procedure.—Weigh out the normal weight of sample and invert it as described on page 293, *b*.   To the inverted solution in a 100-cc. flask add a few drops of phenolphthalein and strong sodium hydroxide solution until slightly pink; add a drop of dilute hydrochloric acid in excess, cool, make up to the mark, and filter if necessary.   Fill the jacketed tube and heat fairly rapidly to about 80°C.   Place the tube in the trough of the saccharimeter, first examining it to see that no air bubbles are present, and heat steadily to 87°.   Take the average of five or six rapid readings at this point, keeping the tube in the instrument no longer than is absolutely necessary.   The slower heating when nearing the desired temperature is advisable in order to avoid troublesome convection currents.   It is usually best to heat to several degrees above 87°, not above 90°, and take the readings when the solution *cools* to 87° rather than to read it while the solution is heating.   Several series of observations should be made on successive fillings of the tube instead of spending time trying to find an exact end point, on account of the greater difficulty usually experienced in reading.   Readings can usually be taken between 86° and 88°.

Calculation of Commercial Glucose.—On account of the variable composition of commercial glucose, its specific rotation

[1] *J. Am. Chem. Soc.*, **1908**, 1790.

ranging from 130 to 154° for the anhydrous substance, it is obviously impossible to calculate the amount exactly from the saccharimeter reading. It is therefore customary to report the percentage in terms of some arbitrary standard. In the "Official Methods" of the Association of Official Agricultural Chemists, it is assumed that the normal weight of sirupy commercial glucose polarizes +175°. The action of the acid during inversion and the expansion of the solution at 87° reduce this value to +163°.[1] The corresponding factors for the commercial glucose solids are +211° and +196°.[2] Hence the calculation of glucose solids may be made by the formula:

$$G = \frac{100S}{196},$$

where G is the percentage of glucose and S the reading of the saccharimeter at 87°. The result may be recalculated in terms of commercial glucose of any Baumé reading desired. It should be stated in the report what factor was used in the calculation. In the absence of any appreciable amount of invert sugar the formula, $G = \frac{(a - S)100}{211}$, in which a = direct polarization, normal solution; and S = percentage of cane sugar, may be used. As noted more fully under Honey, page 338, the determination of commercial glucose by this method in such products as molasses, sirups, and honey, is only approximate, because these materials usually contain dextrins and gums that render them slightly dextrorotatory after inversion, even in the absence of commercial glucose.

CARBOHYDRATES OTHER THAN SUGARS

Determination of Starch. *General Discussion.*—There is no entirely satisfactory method for determining starch in food products. The difficulties encountered in the hydrolytic methods, those depending upon the conversion of starch to soluble carbohydrates by the action of acids or of enzymes, are many. The acid acts on matter other than starch; prepared enzymes differ in hydrolytic power and show distinct variability

[1] LEACH: U. S. Dept. Agr., *Bur. Chem. Bull.* **81**, p. 74.
[2] LATHROP: *J. Assoc. Off. Agr. Chem.*, **1925**, 714.

in their action on starch; malt diastase exhibits varying hydrolytic action on hemicelluloses as well as on starch.

Of the non-hydrolytic methods that of Rask[1] is applicable especially to cereals, whole wheat, and bran. It depends upon the solubility of the starch in cold and relatively concentrated hydrochloric acid. The starch is dispersed into a clear or slightly opalescent but filterable solution from which it can be precipitated quantitatively by alcohol, collected on a filter and weighed. For a good critical discussion of methods for starch determination see Sullivan.[2] The two methods that are described here are the ones that have been largely used in the past, and for results of a fair degree of accuracy, comparable with published analyses, will serve reasonably well.

In the case of nearly pure starch, it can be determined by hydrolysis with acid, as represented by the equation

$$
\underset{\text{Starch}}{\overset{(162)}{C_6H_{10}O_5}} + H_2O \rightarrow \underset{\text{Dextrose}}{\overset{(180)}{C_6H_{12}O_6}},
$$

100 parts of dextrose corresponding to 90 parts of starch. In most vegetable food products, however, the starch is accompanied by pentosans and various hemicelluloses that yield reducing sugars upon hydrolysis, so that if exact results are desired the diastase method should be employed. The method of direct acid hydrolysis has the advantage of being much quicker and easier of execution and is often sufficiently accurate for the purpose.

a. Direct Acid Hydrolysis.—Weigh out from 2 to 5 grams of the sample, depending upon the amount of starch present, and if much fat is present, wash on a good quality filter paper with five successive portions of 10 cc. each of ether. Allow the ether to evaporate from the residue and then wash it with 10 per cent alcohol to free it from soluble carbohydrates. Test the final washings by evaporation on the water bath and note the presence of an appreciable residue. One hundred fifty cubic centimeters of alcohol will usually be enough for washing. Transfer the residue to a 500-cc. graduated flask with 200 cc. of water, add 20 cc.

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[1] *J. Assoc. Off. Agr. Chem.,* **1927,** 108; "Official Methods," **1935,** p. 213.

[2] *Ind. Eng. Chem., Anal. Ed.,* **1935,** 311; *J. Assoc. Off. Agr. Chem.,* **1935,** 621.

of hydrochloric acid (sp. gr. 1.125), place a funnel in the neck of the flask to prevent evaporation, and heat in a boiling water bath for $2\frac{1}{2}$ hours. Cool, nearly neutralize with sodium hydroxide, and make up to 500 cc. Filter and determine the dextrose in an aliquot part of the filtrate, using one of the reducing sugar methods described on pages 263 to 274. The weight of dextrose multiplied by 0.90 gives the weight of starch.

b. Determination with Diastase.—The starch may be separated from the pentosans, which cause the high results of the preceding method, by digestion with diastase, which converts the starch into the soluble products, dextrin and maltose.

Procedure.—Treat 2 to 5 grams of the sample with ether and dilute alcohol as in the preceding method. Transfer the residue with 50 cc. of water to a beaker and heat slowly to boiling or immerse the beaker in boiling water until the starch gelatinizes, stirring constantly to avoid the formation of lumps. Fifteen minutes heating is usually sufficient. Cool to 55°C., add 20 cc. of malt extract[1] and keep the solution within 2° of this temperature for 1 hour. Heat again to boiling to gelatinize any remaining starch granules. Cool to 55°C., add 20 cc. of malt extract and maintain at this temperature for 1 hour, or until a drop of the solution, carefully examined under the microscope, fails to give the iodine reaction for starch. Cool, make up to 250 cc., and filter. The residue on the filter paper should show no signs of starch when examined microscopically. Transfer 200 cc. of the filtrate to a 500-cc. graduated flask, add 20 cc. of hydrochloric acid (sp. gr. 1.125), and carry out the hydrolysis as described in the preceding method.

A blank determination must be carried through in precisely the same manner, using 50 cc. of water and the same amount of malt extract as in the regular procedure, in order to correct for the cupric reducing power of the malt extract itself.

Notes.—The method of determination by diastase gives results that are, in general, lower than those by direct hydrolysis with acid. In food materials that are comparatively high in starch,

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[1] To prepare an active malt extract, digest 10 grams of fresh, finely ground malt several hours at room temperature with 200 cc. of water, shaking occasionally, and filter. Add a few drops of chloroform to prevent the growth of molds.

as ordinary cereals, the difference may be only 3 or 4 per cent; in materials low in starch content but containing large amounts of pentosans, as cocoa shells, or mustard hulls, the results by acid hydrolysis may be 10 to 20 times too high.

Although the factor 0.90 for the calculation of starch from dextrose has been adopted by the Association of Official Agricultural Chemists[1] as standard, it has been shown by various chemists that this theoretical value is never obtained, the actual result being from 95 to 98 per cent of theory, so that more exact results are probably secured by using a somewhat higher factor (0.92 to 0.94) to calculate the starch.

Determination of Pentosans.—The determinations of pentosans and of crude fiber are most commonly made on feeding stuffs, since they represent in general substances indigestible by humans. In examining foods for adulteration these determinations are sometimes useful in pointing out the addition of waste or rejected material, as shells, husks, bran, etc.

The pentosans are commonly determined by distilling with hydrochloric acid under such conditions that the pentoses formed by hydrolysis split up into furfural and water, the method being an elaboration of the qualitative test described on page 261, the conditions being standardized so that a fairly uniform yield of furfural may be obtained. The furfural may be determined by oxidation or precipitation with various reagents, of which phloroglucinol has been the most common. This precipitant, using procedures based on the work of Tollens and his pupils, is the official one of the Association of Official Agricultural Chemists[2] and the one described in detail in previous editions of this book.

Numerous investigators have pointed out objectionable features in the process, a good review being given by Bailey.[3] The distillation is not quantitative, and some furfural may even be destroyed in distilling; the precipitation with phloroglucinol is admittedly inexact, requiring a series of correction factors, and hydrolysis products of hexoses may precipitate in small amount. The precipitate, further, is of variable composition, depending upon the conditions of precipitation. The superiority of bar-

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[1] "Official Methods of Analysis," **1935**, p. 342.
[2] "Official Methods," **1935**, p. 344.
[3] *Ind. Eng. Chem., Anal. Ed.*, **1936**, 389.

bituric acid[1] or, even better, of thiobarbituric acid,[2] has been demonstrated so thoroughly by numerous observers that this method is substituted here for the official one.

Procedure.—Place from 0.5 to 3.0 grams of material, according to its pentosan content, in a 125-cc. distilling flask and add 50 cc. of 12 per cent hydrochloric acid. (This should be 12 per cent by weight of HCl, checked against a standard base.) Distill by steam, having a moderate, constant flow of steam throughout the entire distillation. The temperature, taken by a thermometer in the vapor in the neck of the flask, should be kept between 103 and 105°C. by heating the distilling flask with a burner. Distill until a small sample of the distillate, tested with thiobarbituric acid solution, gives no precipitate or turbidity after standing 5 minutes. To the distillate add a slight excess of thiobarbituric acid in 12 per cent hydrochloric acid at room temperature and allow it to stand overnight. Filter the lemon-yellow precipitate on a Gooch crucible, dry at 105°C., and weigh as furfuralmalonyl-thiourea. The weight of precipitate $\times$ 0.596 = weight of pentosan.

Notes.—The distillation with steam, instead of by direct heating, avoids the decomposition of part of the furfural, which formerly occurred as the acid became more concentrated.[3] The test with thiobarbituric acid, as described, to show the end of the distillation, is better than the usual test with aniline acetate paper since the turbidity is easily distinguished from the color that certain hydrolysis products of hexoses like cellulose give with both thiobarbituric acid and aniline salts. If hexoses are present in small amount the hydroxymethylfurfural that is formed will not interfere with the quantitative pentosan determination, because while it reacts with thiobarbituric acid, the compound formed, in distinction from the reaction with phloroglucinol, is soluble and gives only a color.

Thiobarbituric acid is a somewhat expensive reagent, but only a slight excess is necessary for each determination. It may be purchased from dealers in organic chemicals or prepared[4] with equally good results. The reaction may be expressed:

[1] UNGER and JÄGER: *Ber.*, **1902**, 4440; **1903**, 1222.

[2] DOX and PLAISANCE: *J. Am. Chem. Soc.*, **1916**, 2156.

[3] PERVIER and GORTNER: *Ind. Eng. Chem.*, **1923**, 1167.

[4] *Ber.*, **1904**, 3657.

$$C_4H_3O \cdot CHO + H_2C \overset{\text{CO—NH}}{\underset{\text{CO—NH}}{\diagup}} CS \rightarrow$$

Furfural   Thiobarbituric acid

$$C_4H_3O \cdot CH \cdot C \overset{\text{CO—NH}}{\underset{\text{CO—NH}}{\diagup}} CS + H_2O.$$

Furfuralmalonylthiourea

The factor for calculating the weight of pentosan is based on the following relationships:

$$\text{Pentosan} \rightarrow \text{furfural} + 2H_2O$$

$$\frac{\text{furfural} + 2H_2O}{\text{furfuralmalonylthiourea}} = 0.596$$

Weight of pentosan = weight of furfuralmalonylthiourea

$$\times\ 0.596.$$

Determination of Crude Fiber.—By the term "crude fiber" is ordinarily meant in agricultural and food analysis the organic residue, consisting largely of cellulose, that is left after the other carbohydrates and the proteins have been removed by successive treatments with boiling acid and alkali. The method that has been adopted by the Association of Official Agricultural Chemists as official is the one devised by Henneberg, and usually called the "Weende" method from the experiment station of the name near Göttingen where it was originally employed.[1]

Reagents.—a. 1.25 Per Cent Sulphuric Acid Solution.—Contains 1.25 grams of sulphuric acid (H_2SO_4) per 100 cc.

b. 1.25 Per Cent Sodium Hydroxide Solution.—Contains 1.25 grams of sodium hydroxide (NaOH) per 100 cc., free, or nearly so, from sodium carbonate.

The strength of these solutions must be accurately checked by titration.

Asbestos.—First digest on the steam bath overnight with 5 to 10 per cent sodium hydroxide and thoroughly wash with hot water; then digest overnight with 5 to 10 per cent hydrochloric

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[1] The method given here is essentially the same. In cases of controversy the exact details of the official method should be followed ("Official Methods," **1935**, p. 340).

acid and again wash thoroughly with hot water; next ignite completely at a bright-red heat.

Procedure.—Extract 2 grams of the dry material with ordinary ether, or use the residue from the ether extract determination and transfer the residue, together with 0.5 gram of asbestos, to a 700-cc. Erlenmeyer flask. Add 200 cc. of boiling sulphuric acid, *a*, to the contents of the flask, connect with a reflux condenser, and bring to boiling immediately. It is important that the contents of the flask come to boiling within 1 minute after heat is applied. Continue the boiling gently for 30 minutes. It will be found best to rotate the flask with the hand about every 5 minutes in order thoroughly to mix the charge. Care should be taken to keep the sides of the flask above the solution free from the sample. Remove the flask at the expiration of the 30 minutes and immediately filter through linen in a fluted funnel and wash with boiling water until the washings are no longer acid.

Next wash the charge and adhering asbestos back into the flask with 200 cc. of boiling sodium hydroxide solution, *b*, using a 200-cc. wash bottle. (The transfer of the sample to the original container is easily accomplished by this means.) Then connect the flask with the reflux condenser and boil gently for exactly 30 minutes. Filter directly into a Gooch crucible, which has been prepared previously with a thin but close layer of ignited asbestos. Employ suction and wash the contents thoroughly with hot water and then with about 15 cc. of 95 per cent alcohol.

Dry the crucible and contents to constant weight at 110°C. After weighing, incinerate the contents of the crucible at a dull-red heat until the carbonaceous matter has been removed—20 minutes is usually sufficient. Cool in a desiccator and weigh. The loss in weight is taken as crude fiber.

Notes.—The details of the method must be closely followed in order to secure consistent results. For a careful study of the sources of error consult the paper by Bidwell and Bopst.[1] Of major importance are the following: The material should be as finely ground as possible, since with coarse particles the results may be much too high. In general the substance should be ground so that it will all pass through a 40-mesh sieve, or at least through a sieve with round holes 1 mm. in diameter. (With some materials nothing finer than a 20-mesh sieve can be used.)

[1] *J. Assoc. Off. Agr. Chem.*, **1921**, 58.

Unless the ether extract is removed before the crude fiber is determined the results will be high, the difference depending upon the amount of fat in the sample.* Watch glasses, funnels, air condensers, or round-bottomed flasks containing cold water may be used in place of the reflux condensers, but in general the results will be higher on account of the concentration of the solutions due to partial evaporation. The linen used for the first filtration should be of such character that while filtration is rapid no solid matter passes through. The linen that has proved most satisfactory has 46 by 50 threads per inch.[1] The threads are of large size as compared to the number of threads per inch and are loosely twisted; 350-mesh copper gauze can be used in place of the linen, and a good quality of filter paper may be used for the acid filtration if used with care. The asbestos inhibits somewhat the action of the acid and materially hastens the filtration, thus avoiding the continued action of the reagents. It is especially advantageous with such substances as cocoa, which tend to clog the crucible during the final filtration and filter very slowly.

The crude fiber obtained in this way is not pure cellulose, but contains distinct proportions of hemicelluloses,[2] pentosans, and nitrogenous substances. These, however, are not sufficient to prevent the results from being reasonably accurate and comparable.

Maple Sirup

Source and Methods of Manufacture.—Maple sirup is an essentially American product, being practically unknown to European countries and having a somewhat limited range on this continent. Its use dates back to the first white settlers in this country who, in all probability, learned its nature and source from the Indians.

It is usually prepared from the sap obtained by tapping the hard or rock maple tree, *Acer saccharum*. One or more holes about ½ in. in diameter and 1 to 3 in. deep, according to the size of the tree, are bored. After cleaning the hole, a metal spout is driven in and from this is hung a bucket to catch the sap.

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[1] Butchers linen or dress linen with about 45 threads to the inch, or No. 40 filtering cloth made by the National Filter Cloth and Weaving Company, or its equivalent, will be found satisfactory.

[2] TOLLENS and DÜRING: *J. Landw.*, **1897**, 79; **1901**, 11.

The composition of the sap varies during the season and with the conditions of weather and sunlight, but it is essentially a dilute solution of sucrose containing traces of invert sugar, malic acid, mineral matter, and albuminoids. The average content of sucrose is 3 per cent, and the ordinary yield of sugar from a single tree during the season is about 3 pounds.

The sirup may be made from the sap by concentration in open kettles or by more modern continuous evaporating apparatus. A great many of the small producers still use the primitive kettle method, in which sap is boiled sometimes all day, fresh sap being added continually and the sirup removed at night. The product obtained by this long boiling is dark colored and quite impure, with a strong flavor. The larger producers use continuous evaporators in which the sap enters at one end and passes gradually from one compartment to another, the sirup being drawn off at the other end. This more rapid concentration produces a lighter colored product. During the evaporation, the proteins are coagulated and skimmed off, and a small amount of insoluble residue or "niter," consisting largely of calcium malate, is deposited. For making sirup the sap is boiled until the sirup weighs 11 lb. to the gallon, corresponding to a specific gravity of 1.325. If thinner than this the sirup is liable to ferment; if the density is above 1.325, sugar will crystallize from the sirup.

Strictly speaking, maple sirup made in this way should be called maple-sap sirup to distinguish it from maple-sugar sirup, made by dissolving the solid product of further evaporation in water.

Forms of Adulteration.—The most common adulterant of maple products is refined or granulated sugar, added as such to the maple sugar, and in the form of sirup to maple sirup. A large part of the sirup sold is of this character, the presence of the cane sugar, which makes up usually the greater part of the mixture, being, however, declared on the label. Brown sugar, refinery sirup, or even molasses may be occasionally used, but these adulterants have too pronounced a flavor to permit their use in large amount. Commercial glucose, which was formerly quite extensively used as an adulterant, is not commonly employed now. Preservatives, especially sodium benzoate, may be present occasionally.

Of late it has been recognized that maple products may be seriously contaminated with poisonous metals, especially lead and zinc. The principal sources of lead are the lead-painted buckets and collecting apparatus, "tin" evaporators and tanks made from low-grade terne plate, and lead-soldered joints. Sap that has partly fermented during collection or storage will also dissolve substantial amounts of lead. The Federal authorities recognize no tolerance for lead in maple products, such as is permitted for example in sprayed fruit, it being regarded as entirely avoidable, and the situation is rapidly being corrected.

Doolittle and Seeker[1] have pointed out a possible adulterant of maple products in muscovado sugar, a brown-colored tropical raw sugar. The following table shows the great similarity between this product and genuine maple sugar:

TABLE 35.—COMPARISON OF MUSCOVADO AND MAPLE SUGARS

Determination	Light Muscovado sugar	Dark Muscovado sugar	Vermont maple sugar
Moisture (per cent.)...............	7.35	7.50	2.80
Ash (per cent.)...................	1.33	1.30	1.10
Polarization, direct (°V.).........	+80.0	+82.4	+84.0
Polarization, invert (°V.).........	−27.0	−26.8	−29.6
Polarization, invert at 86° (°V.)....	0.0	0.0	0.0
Sucrose (per cent.)................	81.4	83.1	85.6
Winton lead number..............	2.08	2.12	2.26

As shown on page 329, however, the composition of the ash is sufficiently different from that of maple sugar to serve to detect the product.

METHODS OF ANALYSIS[2]

Preparation of Sample.—Since many maple sirups contain an appreciable quantity of mineral substances which tend to separate on heating, they are best heated and clarified before making the analysis. The methods are described on page 311.

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **122,** p. 196.

[2] The methods of analysis given here, although described mainly for maple sirup, can be applied equally well to maple sugar by using a corresponding weight, or in some cases more simply by dissolving a weighed amount of sugar in water, reboiling as described for sirups, making up to definite volume and using aliquots for the various determinations.

TABLE 36.—SPECIFIC GRAVITY[1] OF SOLUTIONS OF CANE SUGAR AT $\frac{20°}{4°}$C.

Degrees Brix or per cent sugar	Tenths of per cent									
	0	1	2	3	4	5	6	7	8	9
0	0.998234	0.998622	0.999010	0.999398	0.999786	1.000174	1.000563	1.000952	1.001342	1.001731
1	1.002120	1.002509	1.002897	1.003286	1.003675	1.004064	1.004453	1.004844	1.005234	1.005624
2	1.006015	1.006405	1.006796	1.007188	1.007580	1.007972	1.008363	1.008755	1.009148	1.009541
3	1.009934	1.010327	1.010721	1.011115	1.011510	1.011904	1.012298	1.012694	1.013089	1.013485
4	1.013881	1.014277	1.014673	1.015070	1.015467	1.015864	1.016261	1.016659	1.017058	1.017456
5	1.017854	1.018253	1.018652	1.019052	1.019451	1.019851	1.020251	1.020651	1.021053	1.021454
6	1.021855	1.022257	1.022659	1.023061	1.023463	1.023867	1.024270	1.024673	1.025077	1.025481
7	1.025885	1.026289	1.026694	1.027099	1.027504	1.027910	1.028316	1.028722	1.029128	1.029535
8	1.029942	1.030349	1.020757	1.031165	1.031573	1.031982	1.032391	1.032800	1.033209	1.033619
9	1.034029	1.034439	1.034850	1.035260	1.035671	1.036082	1.036494	1.036903	1.037318	1.037730
10	1.038143	1.038556	1.038970	1.039383	1.039797	1.040212	1.040626	1.041041	1.041456	1.041872
11	1.042288	1.042704	1.043121	1.043537	1.043954	1.044370	1.044788	1.045206	1.045625	1.046043
12	1.046462	1.046881	1.047300	1.047720	1.048140	1.048559	1.048980	1.049401	1.049822	1.050243
13	1.050665	1.051087	1.051510	1.051933	1.052356	1.052778	1.053202	1.053626	1.054050	1.054475
14	1.054900	1.055325	1.055751	1.056176	1.056602	1.057029	1.057455	1.057882	1.058310	1.058737
15	1.059165	1.059593	1.060022	1.060451	1.060880	1.061308	1.061738	1.062168	1.062598	1.063029
16	1.063460	1.063892	1.064324	1.064756	1.065188	1.065621	1.066054	1.066487	1.066921	1.067355
17	1.067789	1.068223	1.068658	1.069093	1.069529	1.069964	1.070400	1.070836	1.071273	1.071710
18	1.072147	1.072585	1.073023	1.073461	1.073900	1.074338	1.074777	1.075217	1.075657	1.076097
19	1.076537	1.076978	1.077419	1.077860	1.078302	1.078744	1.079187	1.079629	1.080072	1.080515
20	1.080959	1.081403	1.081848	1.082292	1.082737	1.083182	1.083628	1.084074	1.084520	1.084967
21	1.085414	1.085861	1.086309	1.086757	1.087205	1.087652	1.088101	1.088550	1.089000	1.089450
22	1.089900	1.090351	1.090802	1.091253	1.091704	1.092155	1.092607	1.093060	1.093513	1.093966
23	1.094420	1.094874	1.095328	1.095782	1.096236	1.096691	1.097147	1.097603	1.098058	1.098514
24	1.098971	1.099428	1.099886	1.100344	1.100802	1.101259	1.101718	1.102177	1.102637	1.103097
25	1.103557	1.104017	1.104478	1.104938	1.105400	1.105862	1.106324	1.106786	1.107248	1.107711
26	1.108175	1.108639	1.109103	1.109568	1.110033	1.110497	1.110963	1.111429	1.111895	1.112361
27	1.112828	1.113295	1.113763	1.114229	1.114697	1.115166	1.115635	1.116104	1.116572	1.117042
28	1.117512	1.117982	1.118453	1.118923	1.119395	1.119867	1.120339	1.120812	1.121284	1.121757
29	1.122231	1.122705	1.123179	1.123653	1.124128	1.124603	1.125079	1.125555	1.126030	1.126507
30	1.126984	1.127461	1.127939	1.128417	1.128896	1.129374	1.129853	1.130332	1.130812	1.131292
31	1.131773	1.132254	1.132735	1.133216	1.133698	1.134180	1.134663	1.135146	1.135628	1.136112
32	1.136596	1.137080	1.137565	1.138049	1.138534	1.139020	1.139506	1.139993	1.140479	1.140966
33	1.141453	1.141941	1.142429	1.142916	1.143405	1.143894	1.144384	1.144874	1.145363	1.145854
34	1.146345	1.146836	1.147328	1.147820	1.148313	1.148805	1.149298	1.149792	1.150286	1.150780
35	1.151275	1.151770	1.152265	1.152760	1.153256	1.153752	1.154249	1.154746	1.155242	1.155740
36	1.156238	1.156736	1.157235	1.157733	1.158233	1.158733	1.159233	1.159733	1.160233	1.160734
37	1.161236	1.161738	1.162240	1.162742	1.163245	1.163748	1.164252	1.164756	1.165259	1.165764
38	1.166269	1.166775	1.167281	1.167786	1.168293	1.168800	1.169307	1.169815	1.170322	1.170831
39	1.171340	1.171849	1.182359	1.172869	1.173379	1.173889	1.174400	1.174911	1.175423	1.175935

[1] According to Dr. F. Plato, *Wiss. Abh. der Kaiserl. Normal Eichungs Komm.*, II, **1900**, 153.

TABLE 36.—Specific Gravity of Solutions of Cane Sugar at $\frac{20°}{4°}$C.

(Continued)

Degrees Brix or per cent sugar	Tenths of per cent									
	0	1	2	3	4	5	6	7	8	9
40	1.176447	1.176960	1.177473	1.177987	1.178501	1.179014	1.179527	1.180044	1.180560	1.181076
41	1.181592	1.182108	1.182625	1.183142	1.183660	1.184178	1.184696	1.185215	1.185734	1.186253
42	1.186773	1.187293	1.187814	1.188335	1.188856	1.189379	1.189901	1.190423	1.190946	1.191469
43	1.191993	1.192517	1.193041	1.193565	1.194090	1.194616	1.195141	1.195667	1.196193	1.196720
44	1.197247	1.197775	1.198303	1.198832	1.199360	1.199890	1.200420	1.200950	1.201480	1.202010
45	1.202540	1.203071	1.203603	1.204136	1.204668	1.205200	1.205733	1.206266	1.206801	1.207335
46	1.207870	1.208405	1.208940	1.209477	1.210013	1.210549	1.211086	1.211623	1.212162	1.212700
47	1.213238	1.213777	1.214317	1.214856	1.215395	1.215936	1.216476	1.217017	1.217559	1.218101
48	1.218643	1.219185	1.219729	1.220272	1.220815	1.221360	1.221904	1.222449	1.222995	1.223540
49	1.224086	1.224632	1.225180	1.225727	1.226274	1.226823	1.227371	1.227919	1.228469	1.229018
50	1.229567	1.230117	1.230668	1.231219	1.231770	1.232322	1.232874	1.233426	1.233979	1.234532
51	1.235085	1.235639	1.236194	1.236748	1.237303	1.237859	1.238414	1.238970	1.239527	1.240084
52	1.240641	1.241198	1.241757	1.242315	1.242873	1.243433	1.243992	1.244552	1.245113	1.245673
53	1.246234	1.246795	1.247358	1.247920	1.248482	1.249046	1.249609	1.250172	1.250737	1.251301
54	1.251866	1.252431	1.252997	1.253563	1.254129	1.254697	1.255264	1.255831	1.256400	1.256967
55	1.257535	1.258104	1.258674	1.259244	1.259815	1.260385	1.260955	1.261527	1.262099	1.262671
56	1.263243	1.263816	1.264390	1.264963	1.265537	1.266112	1.266686	1.267261	1.267837	1.268413
57	1.268989	1.269565	1.270143	1.270720	1.271299	1.271877	1.272455	1.273035	1.273614	1.274194
58	1.274774	1.275354	1.275936	1.276517	1.277098	1.277680	1.278262	1.278844	1.279428	1.280011
59	1.280595	1.281179	1.281764	1.282349	1.282935	1.283521	1.284107	1.284694	1.285281	1.285869
60	1.286456	1.287044	1.287633	1.288222	1.288811	1.289401	1.289991	1.290581	1.291172	1.291763
61	1.292354	1.292946	1.293539	1.294131	1.294725	1.295318	1.295911	1.296506	1.297100	1.297696
62	1.298291	1.298886	1.299483	1.300079	1.300677	1.301274	1.301871	1.302470	1.303068	1.303668
63	1.304267	1.304867	1.305467	1.306068	1.306669	1.307271	1.307872	1.308475	1.309077	1.309680
64	1.310282	1.310885	1.311489	1.312093	1.312699	1.313304	1.313909	1.314515	1.315121	1.315728
65	1.316334	1.316941	1.317549	1.388157	1.318766	1.319374	1.319983	1.320593	1.321203	1.321814
66	1.322425	1.323036	1.323648	1.324259	1.324872	1.325484	1.326097	1.326711	1.327325	1.327940
67	1.328554	1.329170	1.329785	1.330401	1.331017	1.331633	1.332250	1.332868	1.333485	1.334103
68	1.334722	1.335342	1.335961	1.336581	1.337200	1.337821	1.338441	1.339063	1.339684	1.340306
69	1.340928	1.341551	1.342174	1.342798	1.343421	1.344046	1.344671	1.345296	1.345922	1.346547
70	1.347174	1.347801	1.348427	1.349055	1.349682	1.350311	1.350939	1.351568	1.352197	1.352827
71	1.353456	1.354087	1.354717	1.355349	1.355980	1.356612	1.357245	1.357877	1.358511	1.359144
72	1.359778	1.360413	1.361047	1.361682	1.362317	1.362953	1.363590	1.364226	1.364864	1.365501
73	1.366139	1.366777	1.367415	1.368054	1.368693	1.369333	1.369973	1.370613	1.371254	1.371894
74	1.372536	1.373178	1.373820	1.374463	1.375105	1.375749	1.376392	1.377036	1.377680	1.378326
75	1.378971	1.379617	1.380262	1.380909	1.381555	1.382203	1.382851	1.383499	1.384148	1.384796
76	1.385446	1.386096	1.386745	1.387396	1.388045	1.388696	1.389347	1.389999	1.300651	1.391303
77	1.391956	1.392610	1.393263	1.393917	1.394571	1.395226	1.395881	1.396536	1.397192	1.397848
78	1.398505	1.399162	1.399819	1.400477	1.401134	1.401793	1.402452	1.403111	1.403771	1.404430
79	1.405091	1.405752	1.406412	1.407074	1.407735	1.408398	1.409061	1.409723	1.410387	1.411051

TABLE 36.—SPECIFIC GRAVITY OF SOLUTIONS OF CANE SUGAR AT $\frac{20°}{4°}$C.

(Concluded)

Degrees Brix or per cent sugar	Tenths of per cent									
	0	1	2	3	4	5	6	7	8	9
80	1.411715	1.412380	1.413044	1.413709	1.414374	1.415040	1.415706	1.416373	1.417039	1.417707
81	1.418374	1.419043	1.419711	1.420380	1.421049	1.421719	1.422390	1.423059	1.423730	1.424400
82	1.425072	1.425744	1.426416	1.427089	1.427761	1.428435	1.429109	1.429782	1.430457	1.431131
83	1.431807	1.432483	1.433158	1.433835	1.434511	1.435188	1.435866	1.436543	1.437222	1.437900
84	1.438579	1.439259	1.439938	1.440619	1.441299	1.441980	1.442661	1.443342	1.444024	1.444705
85	1.445388	1.446071	1.446754	1.447438	1.448121	1.448806	1.449491	1.450175	1.450860	1.451545
86	1.452232	1.452919	1.453605	1.454292	1.454980	1.455668	1.456357	1.457045	1.457735	1.458424
87	1.459114	1.459805	1.460495	1.461186	1.461877	1.462568	1.463260	1.463953	1.464645	1.465338
88	1.466032	1.466726	1.467420	1.468115	1.468810	1.469504	1.470200	1.470896	1.471592	1.472289
89	1.472986	1.473684	1.474381	1.475080	1.475779	1.476477	1.477176	1.477876	1.478575	1.479275
90	1.479976	1.480677	1.481378	1.482080	1.482782	1.483484	1.484137	1.484890	1.485593	1.486297
91	1.487002	1.487707	1.488411	1.489117	1.489823	1.490528	1.491234	1.491941	1.492647	1.493355
93	1.494063	1.494771	1.495479	1.496188	1.496897	1.497606	1.498316	1.499026	1.499736	1.500447
93	1.501158	1.501870	1.502582	1.503293	1.504006	1.504719	1.505432	1.506146	1.506859	1.507574
94	1.508289	1.509004	1.509720	1.510435	1.511151	1.511868	1.512585	1.513302	1.514019	1.514737
95	1.515455	1.516174	1.516893	1.517612	1.518332	1.519051	1.519771	1.520492	1.521212	1.521934
96	1.522656	1.523378	1.524100	1.524823	1.525546	1.526269	1.526993	1.527717	1.528441	1.529166
97	1.529891	1.530616	1.531342	1.532068	1.532794	1.533521	1.584248	1.534976	1.535704	1.536432
98	1.537161	1.537889	1.538618	1.539347	1.540076	1.540806	1.541536	1.542267	1.542998	1.543730
99	1.544462	1.545194	1.545926	1.546659	1.547392	1.548127	1.548861	1.549595	1.550329	1.551064
100	1.551800	....	....	....	....	....	....	....	....	....

For Water Determination.—If the sample is perfectly clear use it directly. If sugar crystals are present redissolve them by heating. If suspended matter or sediment is present filter the sample through cotton wool.

For Other Determinations.—If sugar crystals are present redissolve them by heating. If other sediment is present shake the sirup thoroughly, transfer approximately 100 cc., with its suspended matter, to a casserole or beaker, add one-fourth its volume of water and heat over a flame. When the temperature of the boiling sirup approaches 104°C., draw off about 1 cc. in a small, thin pipette and cool to room temperature in running water. Wipe off the pipette, let a few drops run out, then place a few drops on the refractometer, and determine the solids content. Repeat from time to time if necessary until a value of 65.4 per cent solids is obtained $(n_D^{20} = 1.4521)$. Filter hot

through a rapidly acting filter and adjust the filtrate to 65.0 ($\pm$ 0.5) per cent solids by adding the needed amount of water.

Water (Solids).—As with saccharine products in general, three methods are available for determining the water content.

a. Direct Drying.—Spread about 10 to 15 grams of clean, ignited quartz sand in a flat-bottomed platinum dish, add a short stirring rod, and weigh. Add 2 to 5 grams of the sirup and enough water so that it may be thoroughly mixed with the sand. Dry on the water bath with frequent stirring and finally for 7 to 8 hours in the oven at 100°C., or until the loss in weight during 1 hour does not exceed 3 mg.

Note.—The method described, although tedious as compared with those mentioned below, gives results of reasonable accuracy with maple sirup. With food products, however, containing notable amounts of levulose, as honey, molasses, and jams, the results are always too low on account of the partial decomposition of that sugar (see also page 524). With such products the drying should be done in a vacuum oven at a temperature not exceeding 70°C., for which reason it is usually more convenient, although less accurate, to employ one of the indirect methods.

b. From the Specific Gravity.—Weigh out 20 grams of the sirup, conveniently in a sugar dish (page 289) and transfer to a 100-cc. flask. Dissolve in water and make up to the mark. Determine the specific gravity of the solution with a pykhometer, at 20°C., calculate to $\frac{20°}{4°}$C. and note the corresponding percentage of solids from Table 36, page 309. Calculate the solids in the original sirup by the formula

$$P = \frac{VDS}{W},$$

where P = per cent of solids in the undiluted sample; V = volume of diluted solution; D = specific gravity of diluted solution; S = per cent of solids from the table, and W = weight of sample taken. The water content is found by subtracting the total solids from 100.

If the specific gravity is taken at some temperature other than 20°C., the corresponding percentage of solids may be corrected by Table 37, on page 313, which, although for hydrometer correction,

may be used here. See also Table 1, under General Methods, page 6, if it is desired to convert the specific gravity determined at $\frac{t^\circ}{t^\circ}$ to $\frac{t^\circ}{4^\circ}$.

In commercial work, for rapid testing, the solids content is often determined by a hydrometer. The form most commonly

TABLE 37.—TEMPERATURE CORRECTIONS FOR CHANGING SUGAR PERCENTAGES TO CORRESPONDING VALUES AT 20°C.[1]

Temperature, °C.	Observed per cent of sugar													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract from observed per cent													
13.0	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14.0	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15.0	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16.0	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17.0	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
18.0	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19.0	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.80
	Add to observed per cent													
21.0	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08
22.0	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23.0	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24.0	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25.0	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26.0	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27.0	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28.0	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29.0	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30.0	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81

[1] *U. S. Bur. of Standards Circ.* **19.**

used is the so-called "Brix spindle," graduated to read directly in per cent of sucrose. (Note that the Brix reading is in *per cent by weight*, grams/100 grams.) These were formerly standardized at 17.5°C., but 20°C. is now used. If the reading is made at some temperature other than 20°, which is permissible within a few degrees, the value may be corrected by Table 37.

TABLE 38.—Per Cent of Sucrose in Sugar Solutions Corresponding to Readings of the Abbe Refractometer at 20°C.[1]

N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent
1.3330	0.0	1.3598	17.5	1.3902	35.0	1.4253	52.5
1.3337	0.5	1.3606	18.0	1.3911	35.5	1.4264	53.0
1.3344	1.0	1.3614	18.5	1.3920	36.0	1.4275	53.5
1.3351	1.5	1.3622	19.0	1.3929	36.5	1.4285	54.0
1.3359	2.0	1.3631	19.5	1.3939	37.0	1.4296	54.5
1.3367	2.5	1.3639	20.0	1.3949	37.5	1.4307	55.0
1.3374	3.0	1.3647	20.5	1.3958	38.0	1.4318	55.5
1.3381	3.5	1.3655	21.0	1.3968	38.5	1.4329	56.0
1.3388	4.0	1.3663	21.5	1.3978	39.0	1.4340	56.5
1.3395	4.5	1.3672	22.0	1.3987	39.5	1.4351	57.0
1.3403	5.0	1.3681	22.5	1.3997	40.0	1.4362	57.5
1.3411	5.5	1.3689	23.0	1.4007	40.5	1.4373	58.0
1.3418	6.0	1.3698	23.5	1.4016	41.0	1.4385	58.5
1.3425	6.5	1.3706	24.0	1.4026	41.5	1.4396	59.0
1.3433	7.0	1.3715	24.5	1.4036	42.0	1.4407	59.5
1.3441	7.5	1.3723	25.0	1.4046	42.5	1.4418	60.0
1.3448	8.0	1.3731	25.5	1.4056	43.0	1.4429	60.5
1.3456	8.5	1.3740	26.0	1.4066	43.5	1.4441	61.0
1.3464	9.0	1.3749	26.5	1.4076	44.0	1.4453	61.5
1.3471	9.5	1.3758	27.0	1.4086	44.5	1.4464	62.0
1.3479	10.0	1.3767	27.5	1.4096	45.0	1.4475	62.5
1.3487	10.5	1.3775	28.0	1.4107	45.5	1.4486	63.0
1.3494	11.0	1.3784	28.5	1.4117	46.0	1.4497	63.5
1.3502	11.5	1.3793	29.0	1.4127	46.5	1.4509	64.0
1.3510	12.0	1.3802	29.5	1.4137	47.0	1.4521	64.5
1.3518	12.5	1.3811	30.0	1.4147	47.5	1.4532	65.0
1.3526	13.0	1.3820	30.5	1.4158	48.0	1.4544	65.5
1.3533	13.5	1.3829	31.0	1.4169	48.5	1.4555[2]	66.0
1.3541	14.0	1.3838	31.5	1.4179	49.0	1.4570	66.5
1.3549	14.5	1.3847	32.0	1.4189	49.5	1.4581	67.0
1.3557	15.0	1.3856	32.5	1.4200	50.0	1.4593	67.5
1.3565	15.5	1.3865	33.0	1.4211	50.5	1.4605	68.0
1.3573	16.0	1.3874	33.5	1.4221	51.0	1.4616	68.5
1.3582	16.5	1.3883	34.0	1.4231	51.5	1.4628	69.0
1.3590	17.0	1.3893	34.5	1.4242	52.0	1.4639	69.5

[1] Schönrock: Z. Ver. deut. Zucker-Ind., 1911, 421.
[2] The values above 66.0 per cent are taken from Main's table, Intern. Sugar J., 9, 481.

Table 38.—Per Cent of Sucrose in Sugar Solutions Corresponding to Readings of the Abbe Refractometer at 20°C.—(*Concluded*)

N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent	N_D^{20}	Sucrose, per cent
1.4651	70.0	1.4749	74.0	1.4850	78.0	1.4954	82.0
1.4663	70.5	1.4762	74.5	1.4863	78.5	1.4967	82.5
1.4676	71.0	1.4774	75.0	1.4876	79.0	1.4980	83.0
1.4688	71.5	1.4787	75.5	1.4888	79.5	1.4993	83.5
1.4700	72.0	1.4799	76.0	1.4901	80.0	1.5007	84.0
1.4713	72.5	1.4812	76.5	1.4914	80.5	1.5020	84.5
1.4725	73.0	1.4825	77.0	1.4927	81.0	1.5033	85.0
1.4737	73.5	1.4838	77.5	1.4941	81.5	....	....

Note.—It should be observed that the table on page 309, as is true also of the Brix reading, is based on the specific gravity of sucrose alone. It can be used for the determination of other pure sugars, since these do not differ greatly in specific gravity from equal concentrations of sucrose, but in the determination of total solids in impure products, containing a considerable proportion of non-sugars, the results are not strictly accurate. In the case of such low-grade saccharine products as molasses, for instance, the determination by this method is only an approximation.

c. By the Refractometer.—Determine the refractive index of the sirup by the Abbe refractometer, as described on page 9, correcting, if necessary, for any deviation of temperature from 20°C. (For low concentrations of sugar the immersion refractometer may often be employed to advantage.) The average of three independent settings should be taken, and unless the instrument is known to be in absolute adjustment it should be tested and any correction determined by readings on distilled water. The necessary data for calculating the water will be found in Tables 38, 39 and 40, pages 314, 316, and 317.

The International Sugar Commission in 1936[1] adopted a revised refractometric table with more precise values than those in use previously, based on the values obtained by Landt (*loc. cit.*) with a new refractometer giving refractive indexes of sugar solutions to five decimals. For food analyses, however, where mixtures of different sugars and impurities such as mineral salts

[1] *Intern. Sugar J.*, **1937**, 228.

TABLE 39.—PER CENT OF SUCROSE IN SUGAR SOLUTIONS CORRESPONDING TO READINGS OF THE ZEISS IMMERSION REFRACTOMETER AT 20°C.[1]

Scale reading,[2] 20°C.	$n_D{}^{20}$	Sucrose, per cent	Scale reading, 20°C.	$n_D{}^{20}$	Sucrose, per cent	Scale reading, 20°C.	$n_D{}^{20}$	Sucrose, per cent
14.47	1.33299	.0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

[1] Calculated by J. A. Mathews from the data of SCHÖNROCK-LANDT: *Z. Ver. deut. Zucker-Ind.*, **1933**, 692.

[2] The scale readings refer only to the scale of arbitrary units proposed by Pulfrich (see p. 17). If some other arbitrary scale is on the instrument used the readings must be converted to refractive indexes before the table is used.

TABLE 40.—CORRECTION TABLE FOR DETERMINING SUCROSE IN SUGAR SOLUTIONS BY EITHER THE ABBE OR IMMERSION REFRACTOMETER WHEN READINGS ARE MADE AT TEMPERATURES OTHER THAN 20°C.[1]

Temperature, °C.	Sugar, per cent										
	5	10	15	20	25	30	40	50	60	70	80
	To be subtracted from the per cent of sucrose										
15	0.30	0.31	0.32	0.34	0.35	0.35	0.37	0.38	0.39	0.39	0.40
16	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.30	0.31	0.31	0.32
17	0.18	0.19	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.24
18	0.13	0.13	0.13	0.41	0.14	0.15	0.15	0.15	0.16	0.16	0.16
19	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08
	To be added to the per cent of sucrose										
21	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08
22	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.16
23	0.20	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.24
24	0.27	0.28	0.29	0.30	0.30	0.31	0.31	0.32	0.32	0.32	0.32
25	0.35	0.36	0.37	0.37	0.38	0.38	0.39	0.40	0.40	0.40	0.40
26	0.42	0.44	0.44	0.45	0.46	0.46	0.47	0.48	0.48	0.48	0.48
27	0.50	0.51	0.52	0.53	0.54	0.54	0.55	0.56	0.56	0.56	0.56
28	0.58	0.59	0.61	0.61	0.62	0.63	0.64	0.64	0.64	0.64	0.64
29	0.66	0.68	0.69	0.70	0.71	0.71	0.72	0.72	0.72	0.72	0.72
30	0.75	0.76	0.77	0.78	0.79	0.80	0.81	0.81	0.81	0.80	0.80

[1] Calculated by Snyder and Mathews from the temperature coefficients of SCHÖNROCK: *Z. Ver. deut. Zucker-Ind.*, **1911**, 425.

are commonly present, such refinements, although desirable for some sugar-house products, are hardly necessary, four decimal places being commonly sufficient.

Note.—When a refractometer is available it affords the quickest and most convenient method of determining the water content of most saccharine substances. On sirups and other impure sugar solutions, furthermore, it gives results that are much nearer the actual amount of dry substance than those obtained from specific gravity.

With very dark-colored sirups or solutions, difficulty is sometimes experienced in making readings on account of blurring of the border line owing to excessive dispersion. In such cases it is necessary to dilute the solution. Water should not be used

for dilution, however, on account of the errors caused by the difference in contraction of volume between sucrose and the accompanying impurities when dissolved in water.

Tischtschenko[1] has suggested the elimination of this error by diluting the sirup with a sucrose solution of about the same refractive index. Mix weighed quantities of the solution under examination and a solution of pure sugar of about the same strength, and obtain the quantity of dry substance in the former by the following formula: $x = \dfrac{(A + B)C - BD}{A}$, in which $x =$ percentage of dry substance to be found; $A =$ weight in grams of the solution mixed with B; $B =$ weight in grams of pure sugar solution employed in the dilution; $C =$ percentage of dry substance in the mixture A and B obtained from the refractive index; and $D =$ percentage of dry substance in the pure sugar solution obtained from its refractive index.

Polarization.—Use double the normal weight of the sample in 200 cc. and obtain the direct and invert polarization at 20°C. as directed on page 293. Either 5 cc. of alumina cream or 1 to 2 cc. of neutral lead acetate may be used as a clarifier. Polarization of the inverted solution at 87°C. will usually not be necessary unless the direct reading is decidedly higher than the percentage of sucrose, indicating the presence of commercial glucose. On genuine samples it should be lower, owing to a small amount of invert sugar present.

Reducing Sugar.—Dilute 15 cc. of the solution used for polarizing to 50 cc. and determine the reducing sugar by the Munson and Walker method, page 266. Calculate the result as invert sugar, using the column of the table headed "Invert sugar and sucrose, 2 grams total sugar."

Note.—On account of the large proportion of sucrose, determinations of reducing sugar are not accurate unless allowance is made for the reducing action of the Fehling's solution on the sucrose (see page 269).

Ash.—Use 5 grams of sample, heating cautiously over a burner until charring is complete, then transfer to a muffle, and heat at a temperature not exceeding 550°C. When the carbon is nearly all burned out remove the dish from the muffle, allow to cool, moisten the ash with 0.5 to 1.0 cc. of water, evaporate to dryness,

[1] *Z. Ver. deut. Zucker-Ind.*, **1909**, 103.

and return to the muffle. The moistening facilitates the ashing and diminishes the danger of mechanical loss of the fluffy ash. When the carbon is all burned out, cool the dish, add a few cubic centimeters of ammonium carbonate solution, free from non-volatile matter, evaporate to dryness, and return to the muffle for 2 or 3 minutes to reconvert any alkaline earth oxides that may have been formed to carbonates. Determine the water-soluble and water-insoluble ash, and the alkalinity of the water-soluble ash and of the insoluble ash, as described under General Methods, page 25. Express the alkalinity of the ash in each case as the number of cubic centimeters of $0.1N$ acid required for the ash of 1 gram of sample.

Note.—Observe carefully the rate of burning and the character of the ash. Adulterated sirups usually burn more slowly and leave particles of unburned carbon. Pure maple ash is gray to green in color and sometimes has a curious network structure like the veining of a leaf.

Manganese appears to be a fairly constant constituent of maple-sap ash. Sy[1] showed qualitatively its presence in all of 20 genuine samples examined. Riou and Delorme[2] determined the amount colorimetrically and found it to range from 1 to 12 mg. of manganese per 100 grams of sugar. Refined sugar has no ash content: in raw cane sugar the manganese range is from 0 to 0.4 mg. per 100 grams of sugar.

A method depending, as does the ash determination, upon the proportion of non-sugar constituents in the sirup and its reduction by the addition of refined cane sugar, has been suggested by Snell[3] based on determining the electrical conductivity of a solution of the sirup. The method has the distinct advantage of showing a relatively narrow range of values with genuine sirups, the conductivity value being probably the least variable, the ash value next. On account of the special equipment needed the method has not come into so general use as the simpler ash determination.

Lead Number.—When basic lead acetate is added to maple sirup or to a solution of maple sugar, a voluminous precipitate is produced, consisting mainly of the lead salts of malic and other

[1] *J. Franklin Inst.*, **1908**, 249–280.

[2] *Compt. rend.*, **1935**, 1132.

[3] *Ind. Eng. Chem.*, **1913**, 740.

organic acids, of sulphates, chlorides, and albuminous matter. This is so constant a function of genuine maple products that the amount and character of this precipitate serves to distinguish them from products containing refined cane sugar, which gives practically no precipitate under similar conditions. The following methods are based on this action with lead acetate.

Determination of the volume of the precipitate itself produced under definite conditions, rather than its lead content, may even be used as a rough estimate of the character of the sirup.[1]

a. Winton Method.[2]—Weigh out 25 grams of the sample in a sugar dish and transfer to a 100-cc. flask with water. With a pipette add 25 cc. of standard lead subacetate solution,[3] shake, fill to the mark, shake, and allow it to stand for at least 3 hours. Filter through a dry filter, from the clear filtrate pipette 10 cc. into a beaker, add 40 cc. of water and 10 cc. of sulphuric acid (1 part of concentrated acid to 4 parts of water). Mix and add 100 cc. of 95 per cent alcohol. Let stand overnight, filter on a Gooch crucible, wash with 95 per cent alcohol, dry in a water oven and ignite over a Bunsen burner. Ignite gently at first, then at a low redness for 3 minutes, taking care to avoid the reducing cone of the flame. Cool and weigh.

Blank Determination.—Place 25 cc. of the standard lead solution in a 100-cc. flask, add a few drops of acetic acid, and make up to the mark with water. Shake and use 10 cc. for the determination of the lead as directed above.

Calculation.—Subtract the weight of precipitated lead sulphate in the determination from that in the blank, multiply the difference by 0.6829 to obtain the weight of lead, divide by 2.5, and multiply by 100. The result is the "lead number."

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[1] Hortvet: *J. Am. Chem. Soc.*, **1904**, 1523; Sy: *J. Am. Chem. Soc.*, **1908**, 1430.

[2] Winton and Kreider: *J. Am. Chem. Soc.*, **1906**, 1204.

[3] Activate litharge by heating it to 650 to 670°C. for 2½ to 3 hours in a muffle. (The cooled product should be lemon color.) In a 500-cc. Erlenmeyer flask provided with a reflux condenser boil 80 grams of normal lead acetate crystals and 40 grams of the freshly activated litharge with 250 cc. of water for 45 minutes. Cool, filter off any residue, and dilute with recently boiled water to a density of 1.25 at 20°C.

For the Winton lead number dilute one volume of this solution with four volumes of water and filter if not perfectly clear.

Notes.—The advantages in preparing the basic lead acetate for this test from activated litharge are greater uniformity of the reagent, somewhat higher lead values, and more rapid decrease of the Canadian lead number with increasing addition of sucrose. Something of a disadvantage lies in the fact that the range of values for genuine sirups is somewhat greater than with the ordinary reagent. It should be noted also that many of the earlier values, especially of the Winton lead number, were obtained with the non-activated reagent (page 325).

The acid is added in the blank in order to keep the lead from precipitating (as carbonate) when diluted, *i.e.*, to imitate the action of pure sugar. Without its use a negative lead number would be possible, especially if the blank solution were filtered. It should be added only to the blank, since if used in the determination itself it would dissolve the lead precipitate. The lead may be determined as chromate as described on page 458, with a saving of time and alcohol.

b. Canadian Lead Number.[1]—Weigh out such a quantity of the prepared sirup (page 308) as will contain 25 grams of dry matter, transfer to a 100-cc. flask, and make up to the mark. Pipette 20 cc. into a beaker or large test tube, add 2 cc. of basic lead acetate (footnote, page 320; use without dilution), and mix. Allow to stand 2 hours. Filter on a Gooch crucible having a mat of asbestos at least 3 cm. thick, wash four or five times with boiling water, dry at 100°C., and weigh. Multiply the weight of the dry precipitate in grams by 20.

Note.—The method of determining the weight of the lead precipitate itself, rather than the excess of lead remaining in the solution, has the possible advantage that the result, instead of being proportional to the amount of maple sirup in a mixture with granulated sugar, falls off much more rapidly than the maple-sugar percentage. This makes it distinctly more delicate for detecting slight adulterations but, on the other hand, makes it useless for distinguishing compound maple sirups containing a small proportion of maple from imitation sirups containing none. It has, moreover, a somewhat greater variability in genuine sirups.

[1] McGill: Canada Intern. Rev. Dept., *Bull.* **228**, p. 5; Snell: *Ind. Eng. Chem.*, **1913**, 993.

It has been found by Fowler and Snell[1] that washing with cold water, keeping the precipitate covered with water, and avoiding too rapid filtration prevents cracking of the precipitate and gives results much more precise and somewhat higher than those obtained by the above procedure. They suggest that in filtering, before the liquid has quite passed through, the crucible be filled with cold water and the washing be repeated to a total of four times, taking care to prevent the formation of fissures by keeping the precipitate completely covered with water and filtering at a rate of not more than 40 drops per minute. Dry as before.

Malic Acid Value. *a. Calcium Chloride Method.*[2]—Weigh 6.7 grams of the sample in a sugar dish and transfer to a 200-cc. beaker with 15 cc. of water. Add 2 drops of ammonium hydroxide (sp. gr. 0.90); shake, add 1 cc. of a 10 per cent solution of calcium chloride, then 60 cc. of 95 per cent alcohol; cover with a watch glass and heat on the water bath for 1 hour, then turn off the flame and allow the beaker to stand on the bath overnight. Filter through good quality filter paper and wash the precipitate with hot 75 per cent alcohol until the filtrate measures 100 cc.; dry the precipitate and filter, and ignite in a platinum dish. Add 10 to 15 cc. of $0.1N$ hydrochloric acid to the ignited residue, thoroughly dissolve the lime by heating carefully to just below the boiling point; cool, and titrate the excess of acid with $0.1N$ sodium hydroxide, using methyl orange as indicator. Since 1 cc. of $0.1N$ acid = 0.0067 gram of malic acid and 6.7 grams of sample were weighed, one-tenth of the number of cubic centimeters of acid neutralized by the ignited residue expresses the malic acid value. Run blanks with each set of determinations, using the same amount of reagents and subtract the result from the malic acid value obtained.

b. Calcium Acetate Method.[3]—Weigh 6.7 grams of the sample in a sugar dish. Transfer to a 200-cc. beaker with 5 cc. of water. Add 2 cc. of 10 per cent calcium acetate solution and shake. Stir in 100 cc. of 95 per cent alcohol and agitate the solution until the precipitate settles, leaving the supernatant liquid clear. Filter

[1] *Ind. Eng. Chem., Anal. Ed.*, **1929**, 8.

[2] Leach and Lythgoe: *J. Am. Chem. Soc.*, **1906**, 380; Hortvet: *J. Am. Chem. Soc.*, **1906**, 1536; U. S. Dept. Agr., *Bur. Chem. Bull.* **107**, p. 74.

[3] Cowles: *J. Am. Chem. Soc.*, **1908**, 1285.

off the precipitate and wash with 75 cc. of 85 per cent alcohol. Dry the filter paper and ignite in a platinum dish. Add 10 cc. of 0.1N hydrochloric acid and warm gently until all the lime dissolves. Cool and titrate back with 0.1N sodium hydroxide, using methyl orange as indicator. One-tenth of the number of cubic centimeters of 0.1N acid used up is the malic acid number. Run a blank determination and subtract the result obtained from the malic acid number.

Note.—The two methods depend·upon the precipitation of malic acid as calcium malate, only slightly soluble in alcohol. This when ignited yields calcium carbonate, which is titrated with the standard acid. The "value" obtained is not the actual percentage of malic acid, since calcium, either as the chloride or acetate, in the presence of alcohol, will precipitate other organic acids. The results are, however, comparable if done under uniform conditions.

The amount of ammonia added greatly influences the results, and therefore the directions must be followed exactly. The acetate method is shorter, and the blanks obtained are more uniform than in the other. The results obtained average about 0.2 higher than by the calcium chloride method.

Preservatives.—Occasionally, maple sirup is found containing an added preservative. The sirup is usually one with an excessive water content and the preservative commonly employed is sodium benzoate, which may be detected and determined as described on page 103.

Lead.—A simple procedure for the determination of small amounts of lead which is not too delicate for ordinary work and gives fairly good results is the colorimetric or turbidimetric sulphide method.

Procedure.—Ash 25 to 50 grams of the sirup in a capacious porcelain dish, adding 3 to 5 cc. of "ash-aid"[1] solution and charring by cautious heating at the side of the dish. Finish the ashing in the muffle at a temperature below 500°C. Dissolve the ash in a small amount of dilute nitric acid and 1 cc. of phosphoric acid. Filter and make the filtrate, which should be colorless, alkaline with ammonia. Dissolve the precipitated phosphate, which should contain the lead, in 5 to 10 cc. of dilute

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[1] 40 grams of aluminum nitrate and 20 grams of calcium nitrate in 100 cc. of water.

acetic acid. Make up to 50 cc. in a wide Nessler jar. Add 1 cc. of 5 per cent solution of gum arabic, 5 cc. of saturated hydrogen sulphide solution, and compare the color or turbidity with standard lead solutions treated in the same manner.

Note.—The method described is simple but not sufficiently delicate to determine traces of lead. Such are commonly determined colorimetrically by some modification of the so-called "dithizone" method, based on the colored complex that diphenylthiocarbazone forms with lead and other metals. The method is not one to be lightly undertaken, since it requires special precautions and experience in the preparation of lead-free reagents, even to the distilled water used, apparatus of lead-free glass, which must be rinsed with nitric acid, lead-free water, and even chloroform solutions of the dithizone itself every time it is used. A relatively simple modification of the colorimetric dithizone method, which does not require the use of a spectrophotometer and has been used with maple sirup, is described by Perlman.[1]

Interpretation of Analyses

U. S. Standard for Maple Sugar and Sirup.[2]—"*Maple sirup* is sirup made by the evaporation of maple sap or by the solution of maple concrete, and contains not more than thirty-five per cent. (35%) of water and weighs not less than eleven (11) pounds to the gallon (231 cu. in.).

"*Maple sugar, maple concrete*, is the solid product resulting from the evaporation of maple sap or maple sirup."

Composition of Known-purity Samples.—In Table 41 are collected the results of the earlier analyses of pure maple products by Jones,[3] Hortvet,[4] and Winton[5] and a number of analyses made in the Bureau of Chemistry, in 1904 and 1905.[6]

In Table 42 are given the maximum, minimum, and average results of the analysis of 481 samples of maple sirup of known

[1] *Ind. Eng. Chem., Anal. Ed.*, **1938**, 134.

[2] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[3] *Vt. Agr. Expt. Sta.*, 17*th* and 18*th Ann. Repts.*, 1904 and 1905.

[4] *J. Am. Chem. Soc.*, **1904**, 1523.

[5] *J. Am. Chem. Soc.*, **1906**, 1204.

[6] U. S. Dept. Agr., *Bur. Chem. Circ.* **40**.

TABLE 41.—COMPILED RESULTS ON PURE MAPLE PRODUCTS
(Calculated on Original Substance)

Determination	Maple sugar			Maple sirup		
	Max.	Min.	Av.	Max.	Min.	Av.
Water (per cent.)..........	11.0	3.05	......	......	......	......
Direct polarization °V......	87.4	72.6	......	62.2	51.0	......
Invert sugar (per cent.)....	8.37	1.16	......	9.17	0.34	......
Lead number..............	2.48	1.83	2.23	2.03	1.19	1.49
Total ash (per cent.).......	1.32	0.64	0.91	1.01	0.46	0.60
Soluble ash (per cent.).....	0.67	0.33	0.46	0.63	0.21	0.38
Insoluble ash (per cent.)....	0.87	0.20	0.46	0.56	0.14	0.23
Alkalinity of soluble ash....	0.95	0.40	0.63	0.68	0.26	0.50
Alkalinity of insoluble ash..	1.72	0.55	0.94	0.94	0.31	0.54
Ratio of insoluble to soluble ash...................	2.2	0.5	1.00	3.2	0.6	1.7
Malic acid value..........	1.67	0.65	1.01	1.76	0.41	0.78

TABLE 42.—ANALYSES OF MAPLE SIRUP

Determination	Maximum	Minimum	Average
Moisture (per cent.)..................	48.14	24.85	34.22
Sucrose (per cent.)..................	70.46	47.20	62.57
Invert sugar (per cent.)...............	11.01	0.0	1.47
Ash (per cent.).....................	1.06	0.46	0.66
Direct polarization 20°C.............	+ 69.00	+ 42.10	+ 60.64
Invert polarization 20°C.............	− 24.97	− 17.00	− 22.34
Invert polarization 87°C.............	0.0	0.0	0.0

Calculated to moisture-free basis

Total ash (per cent.)..................	1.68	0.68	1.00
Soluble ash (per cent.)................	1.23	0.35	0.63
Insoluble ash (per cent.)..............	1.01	0.23	0.37
Sol. ash ÷ insol. ash.................	3.86	0.53	1.70
Alkalinity of soluble ash	1.22	0.41	0.75
Alkalinity of insoluble ash............	2.08	0.41	0.97
Alk. sol. ash ÷ alk. insol. ash.....	1.83	0.21	0.77
Lead number........................	4.41	1.76	2.70
Malic acid value (calcium chloride).....	1.60	0.29	0.84
Malic acid value (calcium acetate)......	1.82	0.21	1.01

purity collected from the most important maple-producing districts of the United States and from Canada.[1]

A summary of all the leading analyses of genuine sirups is given by Snell and Scott,[2] Table 43.

In Table 44[3] are given the results of analysis by the same methods of some of the common adulterants.

TABLE 43.—SUMMARY OF ANALYSES OF GENUINE MAPLE SIRUPS ALL ON MOISTURE-FREE BASIS

	Number of analyses	Average	Maximum	Minimum	Range in per cent. of average
Total ash..........................	770	0.96	1.68	0.61	111
Soluble ash........................	770	0.59	1.23	0.30	158
Insoluble ash......................	770	0.37	1.01	0.12	241
Soluble ash ÷ insoluble ash...........	655	1.74	3.86	0.53	191
Alkalinity of soluble ash..............	655	0.74	1.22	0.41	109
Alkalinity of insoluble ash............	655	1.00	2.08	0.41	167
Alkalinity soluble ash ÷ alkalinity of insoluble ash......................	655	0.80	1.83	0.21	203
Canadian lead number (5 grams sirup)	456	2.83	6.56	1.37	183
Canadian lead number (5 grams dry matter)..........................	126	3.48	7.50	1.74	166
Winton lead number.................	528	2.62	4.41	1.05	128
Malic acid value (calcium acetate)	1094	0.80	1.60	0.29	164
Conductivity value.................	174	148	230	110	81

In the greater number of samples the two analytical tests that give most readily an idea of the purity of the product are the total ash and the lead number. If the results on a given sample agree with the average values for the pure product given in the preceding tables, it is a fair assumption either that the sample is genuine or that the adulterant is not the usual one of refined cane sugar. Since the addition of refined cane-sugar sirup is the most common adulteration, the lowering of the ash and lead number, together with a corresponding decrease in the solubility

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **134** (1910).
[2] *Ind. Eng. Chem.*, **1914**, 219.
[3] JONES, *Vt. Agr. Expt. Sta. Rept.*, **1905**.

and alkalinity of the ash, will in the majority of cases be enough to point out the adulterant.

Typical instances of this common form of adulteration, taken from the *Notices of Judgment* published under the Federal Food

TABLE 44.—COMMON ADULTERANTS OF MAPLE SIRUP

Sample	Total ash, per cent.	Soluble ash, per cent.	Insol. ash, per cent.	Alk. of sol.ash, cc.	Alk. of insol. ash, cc.	Ratio of soluble ash : insoluble ash	Malic acid value
Brown sugar:							
Dark..............	4.33	2.74	1.59	0.76	2.34	1.7:1	....
Medium............	2.80	2.15	0.65	0.15	1.18	3.3:1	......
Light..............	0.74	0.68	0.06	0.26	0.15	11.3:1	......
Raw cane sugar......	0.59	0.41	0.18	0.32	0.46	2.3:1	......
Filtered sirup from same..............	0.26	0.16	0.10	0.24	0.24	1.6:1	0.35
Beet sugar:							
White..............	0.33	0.31	0.02	0.40	0.02	15.5:1	....
Light..............	0.86	0.78	0.08	0.38	0.28	9.8:1	0.08
Commercial glucose...	0.57	0.45	0.12	0.24	0.18	3.8:1	......

and Drug Act, together with the general conclusion upon which prosecution was based, are given in Table 45 on page 328.

Snell and Scott[1] suggest the following scheme of tests as being sufficient for the rapid testing of sirup and enough to condemn most of the adulterated samples:

Determination	Limits of value	
	Extreme	Ordinary
Refractometer reading.......................	....	....
Conductivity value, 25°C....................	110–230	113–205
Total ash, dry basis........................	0.61–1.68	0.69–1.47
Alkalinity of soluble ash, dry basis...........	41–122	48–109
Canadian lead number, 5 grams sirup, dry basis..	1.37–6.56	1.51–4.55
Winton lead number........................	0.70–2.70	0.76–2.47

The addition of brown sugar instead of granulated sugar is not shown so readily. The dark grades may be detected in some instances by the pronounced flavor they impart to the product,

[1] *Ind. Eng. Chem.*, **1914**, 219.

but the light sugars, which are more commonly used, can be recognized best by the character of their ash. The total ash of the adulterant may not differ greatly from that of the maple product, but the solubility and alkalinity of the ash are quite different, especially when the ratios for these, referred to the soluble and insoluble ash, are calculated.

TABLE 45.—ADULTERATED MAPLE SIRUPS

Determination	A	B	C	D	E
Total solids (per cent)...	64.5	68.4	69.3	67.82	66.5
Total ash (per cent).....	0.16	0.59	0.20	0.075	0.08
Soluble ash (per cent)...	......	0.56	0.12	0.055	0.04
Insoluble ash..........	......	0.03	0.08	0.020	0.04
Ratio soluble: insoluble ash.................	......	19:1	1.63:1	2.75:1	1:1
Alk. sol. ash (cc. $0.1N$ acid)................	......	2.08	1.14	0.025	....
Alk. insol. ash (cc. $0.1N$ acid.................	......	0.60	0.16	0.145	.....
Ratio alk. sol. ash: alk. insol. ash...........	......	3.5:1	0.875:1	0.17:1	.....
Direct polarization (°V)	+60.5	......	+59.0	+64.5	.....
Invert polarization (°V at 20°C.)...........	−20.1	......	−22.2	−23.6	.....
Invert polarization (°V at 87°C.)...........	......		0.0	0.0	....
Sucrose (per cent)......	61.3	......	61.20	66.41	......
Reducing sugar before inversion (per cent)...	......	......	8.15	....	.....
Lead number..........	0.6	0.52	0.38	0.0	0.11

A.—"Cane-sugar sirup greatly in excess of maple sirup."

B.—"Cane-sugar sirup flavored with some constituent of the maple tree unlike pure sap sirup of live trees."

C.—"Fifty per cent cane sirup; 50 per cent maple sirup."

D.—Formula given on can was: Maple sugar, 40 per cent; cane sugar, 60 per cent. The analysis shows it to contain no appreciable quantity of maple sugar.

E.—"Less than 5 per cent of maple sirup."

The lead number and malic acid value of pure maple products are also greatly reduced by the addition of brown sugar. An analysis of the ash itself will give valuable information in doubtful cases. The percentage of sulphur trioxide and the ratio of calcium oxide to potassium oxide is much higher in brown sugar than in maple sugar.

The value of the determinations of solubility and alkalinity of ash in detecting brown sugars is well illustrated in the muscovado sugar mentioned on page 308. These ratios are shown in the following table:

(CALCULATED TO WATER-FREE BASIS)

Determination	Muscovado sugar	Maple sugar
Water-soluble ash (per cent)...................	1.23	0.50
Water-insoluble ash (per cent)................	0.17	0.64
Ratio $\frac{\text{soluble ash}}{\text{insoluble ash}}$........................	7.7:1	0.8:1
Alkalinity of soluble ash (cc. $0.1N$ acid).........	0.11	0.49
Alkalinity of insoluble ash (cc. $0.1N$ acid).......	0.03	1.47
Ratio $\frac{\text{alk. sol. ash}}{\text{alk. insol. ash}}$........................	3.7:1	0.33:1

The addition of commercial glucose is readily shown by the abnormally high direct polarization, especially as compared with the calculated percentage of sucrose, and by a decided dextro-rotation at 87°C. of the inverted solution. With genuine maple sirup or sugar, the direct polarization usually is slightly *less* than the actual content of sucrose on account of the small amount of invert sugar present; with samples containing commercial glucose the direct reading is decidedly *more* than the sucrose percentage. A slight reading, say of 2 or 3°, to the right at 87°C. on the inverted solution, should not be taken as indicating commercial glucose, since this result might easily arise from fermentation of the sample or from partial destruction of the levulose during inversion and heating, thus leaving an excess of dextrose.

HONEY

Honey, while ordinarily considered as being derived from the nectar of flowers, is more exactly described as "a saccharine product gathered by bees." In addition to the floral nectar, which is the chief source of honey, the bees gather also considerable quantities of various saccharine exudations of leaves and plants which, as will be seen later, materially modify the composition of the final product. Certain enzymes, as invertase and diastase, are also present and active in fresh honey, which may cause gradual changes in the relative proportions of the different sugars present.

TABLE 46.—ANALYSES OF AMERICAN AND IMPORTED HONEYS

Determination	Levo-rotatory honeys (92)			Dextro-rotatory honeys (7)			Imported honeys (72)		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
Direct polarization, 20°C. (immediate), °V	−21.9	+ 3.7	−11.24	+24.9	+ 6.7	+14.77	−22.90	− 6.05	−13.34
Direct polarization, 20°C. (constant), °V	−24.8	− 0.3	−14.73	+17.75	+ 3.6	+ 9.43	−24.15	− 8.50	−14.52
Direct polarization, 87°C., °V	+23.7	+ 0.5	+10.15	+35.8	+28.5	+32.20	+17.00	+ 3.20	+10.31
Invert polarization, 20°C, °V	−29.26	− 1.32	−19.16	+14.96	− 2.53	+ 5.47	−26.07	− 8.86	−16.22
Invert polarization, 87°C, °V	+23.21	− 0.66	+ 7.91	+34.98	+20.90	+27.56	+15.40	+ 2.86	+ 9.08
"Difference", °V	33.55	23.32	27.07	23.43	20.02	22.09	28.93	22.77	25.30
Water (per cent.)	26.88	12.42	17.70	17.80	13.56	16.09	27.00	16.05	21.26
Invert sugar (per cent.)	83.36	62.23	74.98	71.69	64.84	66.96	77.56	68.09	72.38
Sucrose (per cent.)	10.01	0.00	1.90	5.28	0.61	3.01	3.98	0.00	0.80
Ash (per cent.)	0.90	0.03	0.18	1.29	0.29	0.81	0.58	0.06	0.21
Dextrin (per cent.)	7.58	0.04	1.51	12.95	6.02	9.70	3.96	0.26	1.24
Undetermined (per cent.)	7.45	0.04	3.73	4.95	1.57	3.43	8.07	0.66	4.11
Free acid as formic (per cent.)	0.25	0.04	0.08	0.19	0.05	0.12	0.43	0.00	0.15
Reducing sugars as dextrose (per cent.)	79.86	59.61	71.08	68.68	62.12	64.15	....	....	....

Honey is one of the earliest forms of saccharine food products, and on account of its agreeable flavor has continued in use even after cheaper sources of sugar have been developed.

Chemically, it is a sirup made up mainly of invert sugar (dextrose and levulose, but not necessarily in equal amounts), with a varying but relatively small proportion of non-sugars, consisting of mineral matter, proteins, dextrins, and usually formic and malic acids. There are usually present also extraneous substances, as pollen from the flowers and wax from the comb.

Sources.—The main source of honey, and the only source of true honey, is the reducing sugar and sucrose in the nectar of flowers, the latter being changed to invert sugar through the action of an inverting enzyme secreted by the bee.

Considerable quantities are derived also from the so-called "honey dew," an exudation which is produced on the surface of the leaves of plants and trees, especially through the agency of the plant aphis. Honey dew differs from floral nectar in the high percentage of ash and dextrins that it contains, being on account of the latter, strongly dextrorotatory. This, as would be expected, has a decided effect in modifying the physical and chemical properties of the resultant honey.

Composition.—The most extended investigation into the composition of American honeys[1] has been made by Browne,[2] from whose work the preceding figures are taken. In the table are included also data on 72 samples of honey imported from Cuba, Haiti, and Mexico, which closely resemble the American honeys.[3]

Forms of Adulteration.—The forms of adulteration ordinarily found consist in the presence of excessive amounts of sucrose, either added as such or introduced by feeding it to the bees; in the admixture of commercial glucose in greater or less quantity; and in the substitution in part of artificial invert-sugar sirups. The presence of too much water is, of course, also an adulteration.

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[1] Numerous analyses of European honey, which differs slightly from the American product, may be found in KÖNIG: "Chemie der menschlichen Nahrungs- und Genussmittel," or WINTON and WINTON: "Structure and Composition of Foods," Vol. IV.

[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **110.**

[3] BRYAN: U. S. Dept. Agr., *Bur. Chem. Bull.* **154,** p. 9.

METHODS OF ANALYSIS

Preparation of the Sample.—If the honey is clear and liquid, it is ready for analysis without further treatment. If it has granulated and sugar has separated, place the bottle in a bath of water at 45 to 50°C. until it is again entirely liquid. Remove any appreciable amount of wax or other foreign matter by straining through cheesecloth in a hot-water funnel or a fine sieve.

Note.—The granulation of honey, which is of common occurrence, is due to the separation in a crystalline mass of the dextrose, leaving in solution an excess of the less readily crystallizable levulose.

Water.—Because of the decomposition of levulose at temperatures above 70°C., the determination of water should not be made by direct drying, unless a suitable vacuum oven, in which the drying may be made at 70° under reduced pressure, not over 100 mm. of mercury, is available (see page 21). Either method *b* or *c* as given on pages 312 and 315 for maple sirup may be employed.

Note.—It should be noted that the tables on pages 309, 314, and 316 are based in each instance upon the values for solutions of pure sucrose. If, as is commonly done, these tables are used with solutions of sugars other than sucrose, the accuracy of the result is necessarily dependent upon the closeness of agreement between the density coefficient or refractive constant of these other sugars and sucrose. With most edible sirups the quicker methods give a sufficiently close approximation, although in the case of honey the divergence is a little greater.[1]

Ash.—Use 5 grams of the sample and determine the ash as directed under General Methods, page 23. A bit of vaseline or a few drops of pure olive oil may be used if desired to prevent spattering and decrease foaming.

Polarization. *a. Direct.*—Use the normal weight of sample and proceed as directed on page 293 using, however, 5 cc. of alumina cream instead of lead subacetate as a clarifier. To obtain the constant rotation, add 2 or 3 drops of ammonia before making up to the mark (see Mutarotation, page 278. Read also the

[1] BRYAN: *J. Am. Chem. Soc.*, **1908,** 1443; SCHNELLER: *J. Assoc. Off. Agr. Chem.,* **1926,** 157; BROWNE: "Handbook of Sugar Analysis," p. 62.

notes on page 295). Polarize the solution at 20°C. Save a portion of this solution for the determination of reducing sugars.

b. Invert.—The inversion, if done with care, may be made by heating as described on page 294, noting that the addition of potassium oxalate is in this case not necessary. Owing, however, to the large proportion of levulose present, it is much better to follow the method of inverting by standing at room temperature overnight, as given on page 293, which likewise explains the suggested use of sodium chloride in the direct polarization. After polarizing the inverted solution at 20°C., measure out 50 cc. into a 100-cc. flask, nearly neutralize the acid and polarize at 87°C., as directed on page 297. (Save a portion of the inverted and neutralized solution for the determination of the reducing sugars after inversion.)

Reducing Sugars.—Pipette 10 cc. of the solution used for direct polarization into a 250-cc. graduated flask, make up to the mark and determine the reducing sugars by one of the reducing sugar methods given on pages 263 to 275. Calculate the result as invert sugar.

To determine the *reducing sugars after inversion*, pipette 25 cc. of the solution prepared for polarization at 87° into a 250-cc. flask, dilute to volume, and determine the reducing sugars as in the preceding paragraph.

Sucrose.—This should be calculated from the difference in percentage of reducing sugars before and after inversion (see page 275) rather than from the polarization. This is because of the error due to the change in specific rotation of levulose in neutral and in acid solution, which may amount to 1 per cent or more, an amount that might be sufficient to condemn unjustly a honey for excessive sucrose content.

Qualitative Tests for Adulterants. Commercial Glucose.— An indication of the presence of commercial glucose may be had from characteristic reactions of its dextrins.

a.[1] To about 5 cc. of glacial acetic acid in a test tube add one drop of honey with a glass rod, heat in a boiling water bath with frequent shaking, then cool. If a white turbidity appears, the test can be repeated several times by adding fresh drops of honey. After the limiting solubility of glucose sirup in acetic

[1] RAIKOW: *Z. anal. Chem.*, **1939**, 40.

acid has been reached, fresh additions of honey cause the acid gradually to clear.

Note.—If a positive test is obtained, the test being quite delicate, the second reaction should be tried.

b.[1] To 5 cc. of honey add an equal volume of water and then add a solution of iodine in potassium iodide, a few drops at a time, with frequent shaking, noting any change in the color of the solution to red or violet. Compare the color with a similar test made on pure honey, using the same amount of iodine solution.

Notes.—The test depends upon the presence in commercial glucose of erythrodextrin, which is one of the intermediate products formed in the acid conversion of starch and gives a red color with iodine. It is limited somewhat by the fact that high converted glucose, in which the conversion has proceeded nearly to the final stage, does not always give a reaction with iodine.

The test may be made more sensitive by precipitating the dextrins of the honey with strong alcohol, dissolving the precipitate in a few cubic centimeters of water and testing with iodine as before.

Artificial Invert Sugar. *Anilin Chloride Test.*[2]—The reagent used, which is best if freshly prepared, is made by adding to 10 cc. of pure anilin 3 cc. of 25 per cent hydrochloric acid.

To make the test mix directly 5 grams of honey in a porcelain dish with 2.5 cc. of the anilin reagent. A bright red color, appearing at once, indicates the presence of artificial invert sugar.

Note.—The test can be applied directly only to light-colored honeys. In the case of darker samples, apply the test to a portion of the ether extract, as described in the following test.

Resorcin Test.[3]—Dissolve 10 grams of honey in 10 cc. of cold water and extract with 20 cc. of ether by gentle mixing. Avoid violent shaking. Decant the ether and evaporate at room temperature; dissolve the residue in 10 cc. of ether. Test 2 cc. of the ethereal solution by the addition of 2 cc. of a freshly prepared 1 per cent solution of resorcinol in concentrated hydrochloric acid. A positive test is indicated by the immediate appearance of a pink

[1] *Z. anal. Chem.*, **1896**, 267.

[2] FEDER: *Analyst*, **1911**, 586.

[3] FIEHE: *Z. Nahr.-Genussm.*, **1908**, 75; BRYAN: U. S. Dept. Agr., *Bur. Chem. Bull.* **154**, p. 15; LAMPITT, HUGHES and ROOKE: *Analyst*, **1929**, 318.

color in the acid layer, within 2 or 3 minutes at the most; this rapidly darkens until after 20 minutes there is a deep cherry-red color at the junction of the acid and ethereal layers. Any color appearing only after 20 minutes should be disregarded, as should also any but a definite cherry-red color.

Evaporate the rest of the ethereal extract to dryness at room temperature and add to the residue 2 cc. of anilin chloride as described in the previous test. In positive tests a pink to orange color appears within 15 minutes. Both tests should be positive before concluding that commercial invert sirup is present. If a faint positive test is given, run a blank test on the reagents. The anilin chloride test is less delicate than the Fiehe test, but at the same time less sensitive to the effect of age and temperature; hence one supplements the other.

Notes.—Both this reaction and the preceding one are tests for *oxymethylfurfural*, a decomposition product of levulose, and are taken as indicating the presence of artificial invert sugar. This is made on a commercial scale by heating sucrose with a small amount of tartaric or citric acid to about 115°C., under which conditions traces of oxymethylfurfural are formed. If the inversion were made with invertase, the tests would be of no use.

Honey that has been boiled or strongly heated for some time, especially if somewhat acid in reaction, will sometimes give a similar reaction, but such high temperatures are seldom used with the commercial product because the appearance and flavor are thereby impaired, the color being much darker. Experimental tests have shown that honey must be heated almost to incipient caramelization to give a good positive reaction. Long storage of honey, over a period of several years, has also been stated to cause a positive reaction. In the case of honeys heated to temperatures that would prevail in the ordinary commercial handling of the product, the two tests described, if used with discrimination, will be found reliable for 10 per cent or more. In doubtful cases it would be worth while also to test for tartaric, sulphuric, and phosphoric acids, small amounts of which might remain in the finished product.

Microscopical Examination.—Dilute a small portion of the honey with three or four times its volume of water and centrifuge. Place the sediment on a clean microscope slide, cover with a cover glass, and examine with a magnification of about 250 diameters.

Detailed descriptions and illustrations of the most commonly occurring pollens, together with an analytical key for their identification, will be found in *Bulletin* 110 of the Bureau of Chemistry. If, as is often desirable, pollen grains from known floral sources are used for comparison, they should be soaked in honey for 24 hours, since the pollen grains tend to alter a little in shape while in the honey, owing to absorption of water.

Note.—The identification of the pollen is of considerable value in determining the correctness of labeling of a sample for which a particular floral source is claimed.

INTERPRETATION OF RESULTS

The Federal standards define honey as "the nectar and saccharine exudations of plants gathered, modified, and stored in the comb by honey bees (*Apis mellifica* and *A. dorsata*); it is levorotatory, contains not more than twenty-five (25) per cent of water, not more than twenty-five hundredths (0.25) per cent of ash, and not more than eight (8) per cent of sucrose."

Cane Sugar.—The average sucrose content of the 99 honeys analyzed by Browne[1] was 1.98 per cent, and of the whole number only two exceeded the Federal standard of 8 per cent, so that this may be considered a liberal allowance and amounts exceeding this should be regarded as added sugar. The addition of cane sugar in such small quantities that the total amount present does not exceed 8 per cent obviously cannot be shown by analysis. The same remarks apply to sucrose added by feeding cane sugar to the bees. The latter practice, however, is not common, being unprofitable commercially.

Commercial Glucose.—This may be added to honey in considerable quantities simply as a cheaper substitute, or used in lesser amounts to modify and improve the color and taste of dark-colored and strongly flavored grades of natural honey. The addition of a comparatively small proportion of glucose prevents the granulation of the honey. For whatever purpose added, the presence and amount should be made known to the purchaser by the label, although this is not required under Federal regulations. The general character of commercial glucose or corn sirup is well shown in the analyses in Table 47.[2]

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **110.**
[2] LATHROP: *J. Assoc. Off. Agr. Chem.*, **1925,** 714.

TABLE 47.—Composition of Commercial Glucose

Sample number	Baumé	By refractometer Moisture, per cent	By refractometer Total solids, per cent	By vacuo drying 70°C Moisture, per cent	By vacuo drying 70°C Total solids, per cent	Sugars before inversion as glucose (by copper), per cent	Sugars after inversion as glucose (by copper), per cent	Sugar after 2½ hrs. boiling in dilute HCl as glucose (by copper), per cent	Total ash, per cent	Alkalinity of ash, c.c. normal acid for 1 gram ash	Sulphur in ash, per cent	P_2O_5 in ash, mg, per 100 grams	K_2O in ash, per cent	Cl in ash, per cent	Acidity per 100 grams, cc. N/10 acid	Before inversion 20°	After inversion 20°	After inversion 87°	Commercial glucose, factor 163, per cent	Commercial glucose solids, factor 196, per cent
1	44.17	15.62	84.38	17.08	82.92	34.98	35.81	83.52	0.287	10.5	3.99	2.97	0.61	24.40	4.0	+178.2	+176.8	+168.0	101.8	85.64
2	43.42	17.83	82.17	18.57	81.43	35.25	36.34	79.76	0.269	10.6	4.24	29.6	0.79	25.90	4.0	+172.8	+169.6	+160.8	98.6	81.97
3	42.98	18.50	81.50	19.70	80.30	35.49	36.40	81.16	0.314	10.8	2.39	28.9	1.79	21.08	8.0	+169.6	+167.6	+157.0	96.3	80.37
4	43.02	18.58	81.42	19.51	80.49	37.37	37.59	81.28	0.290	11.7	2.37	16.7	2.14	21.18	8.0	+166.2	+163.6	+153.0	93.9	78.00
5	43.03	18.45	81.55	19.58	80.42	36.05	37.36	81.20	0.301	13.5	2.68	17.7	2.64	25.31	5.3	+167.2	+165.6	+155.4	95.3	79.22
6	43.63	16.73	83.27	18.34	81.66	35.33	37.01	81.56	0.318	11.4	5.03	23.0	1.80	18.49	6.7	+175.0	+173.4	+163.2	100.1	83.19
7	43.00	18.45	81.55	19.58	80.42	32.95	33.11	81.04	0.409	9.8	4.25	21.7	1.49	21.03	6.7	+175.0	+173.4	+160.4	98.4	81.76
8	42.89	18.58	81.42	19.55	80.45	35.64	35.85	80.96	0.323	10.6	3.34	16.9	3.48	22.79	6.7	+168.6	+167.6	+156.8	96.2	79.93
9	43.58	17.03	82.97	18.63	81.37	37.20	38.15	82.00	0.381	14.7	1.54		3.65	18.30	5.3	+170.0	+167.4	+158.4	97.2	80.75
Average 9 samples	43.30	17.75	82.25	18.94	81.05	35.55	36.28	81.40	0.321	11.5	3.31	23.4	2.04	22.05	6.1	+171.4	+169.4	+159.0	97.50	81.05
Moisture-free basis, vacuo drying 70°C					100.00	43.98	44.76	100.43	0.396	11.5	3.31	28.9	2.04	22.05	7.5	+211.5	+209.1	+196.17	118.5	100.00

For the detection of commercial glucose in honey, especially if present in small amounts, the method of polarizing the invert solution at 87°C., as commonly used for maple sirup and jams, is not suitable. This is due to the fact that genuine honey is nearly always dextrorotatory under these conditions, the values given in the table on page 330 ranging from $+23.21$ to a minimum of -0.66 for the levorotatory honeys alone. For this reason the invert polarization at 87°, unless distinctly more dextrorotatory than the maximum value, should be interpreted with extreme caution and chief reliance placed on the erythrodextrin test on the honey or on the precipitated dextrins.

For the same reason the method of calculating the amount of commercial glucose from the invert polarization at 87°, as described on page 299, can be taken only as an approximation in the case of honey.

Another method of calculation, which gives somewhat better results, is based upon the variation of the invert polarization of the sample from an assumed value for pure honey. For example, taking $-17.5°$ as the average invert polarization (at 20°C.) of the honeys in Table 46 and $+175°$ as the average polarization of commercial glucose (see page 299), let

x = percentage of honey in the sample,

y = percentage of commercial glucose in the sample, and

P = invert polarization for normal weight of sample.

Then

$$x + y = 100$$
$$-0.175x + 1.75y = P,$$

whence

$$y = \frac{P + 17.5}{1.93}.$$

An objection to this method, however, lies in the considerable variation in the invert polarization of genuine honey itself, the extremes of Table 46 showing values of -29.26 to $+14.96$.

A still better method is the one suggested by Browne, based on the difference in the invert polarizations at 20 and 87°. Browne has found that, although the invert polarizations at 20 and 87° are subject to wide variations, the difference between the two polarizations is much more nearly constant, ranging in a large proportion of cases between the rather narrow limits of 23 and 30.

TABLE 48.—POLARIZATION OF HONEY AND GLUCOSE MIXTURES

Sample	Direct polarization, 20°C., °V	Invert polarization, 20°C., °V	Invert polarization, 87°C., °V	Polarization difference (87°-20°), °V	Invert sugar after inversion, per cent.	Pol.-diff. corrected to 77 per cent. invert sugar, °V	Glucose from invert polarization at 87°C., per cent.	Glucose from invert polarization at 20°C., per cent.	Glucose from polarization difference, per cent.
Alfalfa honey	−19.5	−22.66	+ 3.52	26.18	77.84	25.90	2.16	0.00	3.00
Alfalfa honey + 20 per cent. glucose	+19.4	+16.88	+35.82	18.94	70.01	20.83	21.97	17.82	21.98
Hop-vine honey	−12.6	−16.83	+ 9.68	26.51	75.83	26.92	5.94	0.35	0.00
Hop-vine honey + 20 per cent. glucose	+24.9	+ 2.54	+40.74	19.20	68.14	21.70	25.00	20.28	18.72
Basswood honey	− 0.3	− 1.32	+23.21	24.53	70.60	26.75	14.24	8.40	0.00
Basswood honey + 20 per cent. glucose	+ 3.48	+33.94	+51.57	17.63	63.97	21.22	31.64	26.72	20.52
White oak honey	+11.0	+ 5.17	+28.60	23.42	70.44	25.61	17.56	11.23	4.08
White oak honey + 20 per cent. glucose	+43.8	+39.14	+55.88	16.74	63.84	20.20	34.28	29.35	24.35

Since this difference in polarization is due entirely to the decreased rotation of levulose with increased temperature, in other words, is dependent entirely upon the percentage of invert sugar present, the addition of commercial glucose will lower the polarization difference by an amount proportional to the quantity of commercial glucose added, but irrespective of its specific rotation. On account of the variations in water content and non-sugar solids of pure honey, it is best in applying this method of calculation to reduce the polarization difference to a uniform basis of 77 per cent reducing sugars, which is the average amount of invert sugar after inversion in pure honey. Taking the average value found by Browne for the polarization difference in pure honey as 26.7, the expression would become

$$H = \frac{100(P^1 - P) \times 77}{26.7},$$

where H = percentage of pure honey in the sample,

$\quad P^1$ = invert polarization at 87°C.,

$\quad P$ = invert polarization at 20°C.,

and $\quad I$ = percentage of invert sugar after inversion, whence

$$100 - H = \text{percentage of commercial glucose.}$$

It will be seen by examination of the table on page 339 in which Browne has compared these three methods of calculation on known mixtures of commercial glucose with different honeys, both levorotatory and dextrorotatory, that, with the latter especially, the calculation from the polarization difference gives results on the whole closest to the truth. It is apparent, further, that none of the methods is by any means exact and that positive results on a dextrorotatory honey should not condemn it unless confirmed by the qualitative tests. If the sample contains added commercial glucose there will in nearly every case be a noticeable depression of the "polarization difference" (corrected to 77 per cent of invert sugar), and a positive reaction of the honey and of its precipitated dextrins toward iodine.

The following table shows the analytical results given by a honey containing a large (50 per cent) and a small (5 per cent) proportion of commercial glucose.

Invert-sugar Sirup.—The addition of invert-sugar sirup constitutes a form of adulteration that is quite difficult of detection because the analytical constants of the adulterant so closely resemble those of honey itself. The product obtained, for instance, by heating a sugar sirup with 0.1 per cent of citric or tartaric acid is almost identical in chemical composition with honey. Auerbach and Bodlander[1] have suggested the use of the dextrose-levulose ratio to detect artificial honey, since they have found that genuine honey contains an excess of levulose over

TABLE 49.—ANALYSES OF HONEY ADULTERATED WITH COMMERCIAL GLUCOSE

Determination	A Honey + 50 per cent commercial glucose	B Honey + 5 per cent commercial glucose
Direct polarization, 20°C.	+67.00	−11.50
Invert polarization, 20°C.	+65.67	−14.31
Invert polarization, 87°C.	+73.81	+11.66
Polarization difference	8.14	25.96
Invert sugar before inversion, per cent.	53.67	75.74
Invert sugar after inversion, per cent.	54.50	77.80
Water, per cent.	20.52	19.76
Ash, per cent.	0.49	0.22

dextrose, the ratio of the two falling within rather narrow limits. Commercial invert sugar, on account of the method of manufacture, usually contains slightly more dextrose than levulose. Further study of this ratio on a greater number of samples, especially in the case of American honeys, however, has shown it not to be sufficiently sensitive, the range of values for genuine honey being much greater than at first found, so that, although helpful, it is not a positive indication. The most satisfactory tests for the presence of invert sugar sirup in honey depend upon the formation of traces of some characteristic product by the heating with acid. The best known of these are Fiehe's test and the reaction with anilin chloride described on page 334. If both of these tests give a positive result when carefully compared with similar tests on pure honey, it may safely be concluded that artificial invert sugar is present.

[1] *Z. Nahr.-Genussm.*, **1924**, 233.

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CHAPTER VII

COCOA AND CHOCOLATE

Source.—Cocoa, chocolate, and their preparations are derived from the seeds of the *Theobroma Cacao*, a tree that is cultivated in many semitropical countries, especially in South America, the West Indies, Java, and Africa. The seeds or "cocoa beans," as they are commonly called, appear in commerce under various names, derived mostly from the district in which they are grown or the port from which they are shipped. Among the more important varieties are: Ariba (Ecuador), Bahia (Brazil), Caracas (Venezuela), Ceylon, Java, Surinam (Dutch Guiana), Trinidad (West Indies), and St. Thomé (Africa).

The seeds are borne in pods about 6 to 10 in. long and shaped something like an overgrown cucumber. These contain from 25 to 75 of the almond-shaped seeds imbedded in a mass of cellular pulp. The pods are cut open, and the beans, separated from the pulp, are subjected to a sort of fermentation or "sweating" process for several days, during which the temperature rises somewhat. The results of this treatment are the development of the flavor to a considerable extent, the change of color to the familiar rich chocolate tint, and the hardening of the shell. After drying, either in the sun or by artificial heat, the beans are ready for shipment.

Manufacture.—In the manufacture of chocolate the cleaned and sorted beans are roasted at a temperature of 140 to 300°C., thereby developing the aroma of cocoa through changes brought about in the essential oils. Another important result of the roasting is the drying of the shell, facilitating its removal, which is done by a thorough winnowing of the crushed beans. The roasting process is of great importance since it is the chief factor in producing the fine flavor of the chocolate.

The crushed beans, freed from shells, are sometimes sold without further treatment as "cocoa nibs" or "cracked cocoa," but by far the greater part are ground between stones in steam-heated mills. Only a slight warming of the stones by the steam

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coils at the start is required, the heat of grinding and the high fat content being sufficient to keep the material liquid. The resultant thin paste is run into molds and, after hardening, constitutes the ordinary "plain," "bitter," or unsweetened chocolate.

Sweet chocolate is made by mixing powdered sugar and flavoring, usually vanilla, with the warm chocolate paste in a special mill or "melangeur." The mixture is hardened in molds as in the case of the plain chocolate. Cocoa, called also "breakfast cocoa" or "cocoa powder," is prepared by expressing a portion of the fat or "cocoa butter" from the warm cocoa mass in a hydraulic press. The residue left in the press is then crushed, ground, and sifted until it becomes a very fine powder. Alkalies or other chemicals are sometimes added during the process, producing the so-called "Dutch process cocoa" (see page 348). Other optional ingredients, as salt, seasoning, spice, honey, or various food flavoring oils or oleoresins, may also be used under certain restrictions.

Milk chocolate is a somewhat more recent cocoa preparation and is made by adding to the warm chocolate mass either specially prepared condensed milk, dry milk powder, or other commercial milk or skim milk preparations.

The manufacturing process, although largely mechanical, requires skilled supervision to bring about the best results, and the proper blending of the different varieties of cocoa beans in order to yield the special flavor desired can be learned only by long experience. The differing degrees of softness of the chocolate, as required for the various uses to which it is put by the confectioner, are attained by mixing in the necessary proportion of the "butter" expressed in the manufacture of cocoa.

Composition.—The most important constituents of the cocoa bean are the fat, which makes up about half the weight of the bean, protein, starch, theobromine and caffeine, and a peculiar pigment known as "cocoa red." The fat has considerable commercial use in addition to its use by the cocoa manufacturer in blending or softening his products, being quite largely employed in pharmacy. It is not usually regarded as an edible fat when in a pure condition, on account of its indigestibility, although when in the form of chocolate it is stated to be nearly as digestible as milk fat.[1]

[1] ZIPPERER: "The Manufacture of Chocolate," p. 44.

The stimulating effect of cocoa as a beverage is due mainly to the purine bases, commonly spoken of as alkaloids, theobromine and caffeine, from 0.9 to 3.0 per cent of the former and 0.05 to 0.35 per cent of the latter being present. The theobromine is found in the bean in two forms, partly in combination with dextrose and the cocoa red as a glucoside and tannate and partly as free theobromine, having been set free from the glucoside during the fermentation process. Chemically it is closely related to caffeine, of which it is a lower homologue. Theobromine is 3,7-dimethyl-xanthine.

$$
\begin{array}{l}
\mathrm{HN-CO} \\
\quad| \qquad | \qquad \diagup \mathrm{CH_3} \\
\mathrm{OC \quad C-N} \\
\quad| \qquad \| \qquad \diagdown \mathrm{CH,} \\
\mathrm{CH_3N-C-N} \diagup
\end{array}
$$

while caffeine is 1,3,7-trimethyl-xanthine,

$$
\begin{array}{l}
\mathrm{CH_3N-CO} \\
\quad \diagup \qquad | \qquad \diagup \mathrm{CH_3} \\
\mathrm{OC \qquad C-N} \\
\quad | \qquad \diagup\diagup \quad \diagdown \mathrm{CH} \\
\mathrm{CH_3N-C-N} \diagup\diagup
\end{array}
$$

If to a minute quantity of either caffeine or theobromine in a porcelain dish are added a few drops of strong hydrochloric acid and a small crystal of potassium chlorate, and the mixture is evaporated to dryness on the water bath, a reddish yellow or pink spot is left. On moistening this, when cold, with a few drops of ammonia, a beautiful purple color is produced, the so-called "murexide" test.

Composition	Per cent	Composition	Per cent
Moisture	6.3– 8.5	Cocoa red	2.5– 5.0
Fat	46.9–52.1	Ash	2.9– 4.8
Albuminoids	11.6–21.1	Astringent matters }	7.2– 8.6
Cellulose	3.3– 6.6	Cane sugar }	
Alkaloids	0.3– 0.5	Starch	8.7–12.6

The cocoa bean also contains 2 to 5 per cent of a peculiar and interesting pigment, the "cocoa red," which is formed during the drying of the white beans by the action of enzymes on the glucosides. It is not only a prominent factor in determining the peculiar odor and taste of the cocoa bean, but is also of great

importance to the manufacturer, since it determines very largely the color of the finished product. The general composition of the cocoa bean is summarized in the analysis by Zipperer[1] shown in the table on page 345.

A somewhat more detailed analysis, by modern methods, and showing the effect of roasting on the bean, is given in Table 50.[2]

TABLE 50.—ANALYSES OF RAW AND ROASTED COCOA BEANS AND SHELLS

Determination	Raw nibs, per cent	Roasted nibs, per cent	Raw shells, per cent	Roasted shells, per cent
Water..........................	5.13	3.71	8.69	6.01
Total ash.......................	3.05	3.14	11.40	12.04
Ash soluble in water.............	1.39	1.45	3.63	4.24
Ash insoluble in acid............	0.02	0.00	4.59	4.55
Alkalinity of ash, cc. 0.1N acid...	2.35	2.50	5.32	5.35
Theobromine....................	1.03	1.02	0.33	0.39
Caffeine........................	0.42	0.41	0.20	0.21
Other nitrogenous substances.....	11.38	11.56	12.50	12.69
Crude fiber.....................	1.90	2.71	13.41	15.55
Starch by acid hydrolysis........	10.14	10.00	11.35	10.37
Starch by diastase..............	6.93	7.41	4.59	4.37
Other nitrogen-free substances....	18.71	18.39	44.61	45.99
Fat............................	51.45	51.65	4.27	2.68
Total nitrogen..................	2.26	2.29	2.16	2.24
Direct polarization, °V..........	0.0	0.0	+4.0	+5.0
Invert polarization, 20°C., °V.....	0.0	0.0	+4.0	+4.8
Melting point of fat, °C..........	33.0	32.7		
Refractive index, 40°C...........	1.4576	1.4576		
Iodine number..................	36.33	35.61		

A discussion of the differences in composition between the nibs and the shells, and of the composition in relation to the conclusions to be drawn from the analytical results, will be found under the Interpretation of Results.

Forms of Adulteration.—The most common adulterations of *plain* or *bitter chocolate* which have been reported at various times are the addition of foreign starches, as wheat or arrowroot, or the inclusion, either by accident or intentionally, of a portion of the shells. Occasionally a portion of the fat is found to have

[1] *Loc. cit.*

[2] WINTON, SILVERMAN, and BAILEY: *Conn. Agr. Exp. Sta., Ann. Rept.,* **1902,** 268.

been removed, or when the proper amount of fat is found, its constants show that a part of the cocoa butter has been replaced by cheaper substitutes of which there are several on the market, based on the use of coconut oil. In Germany, so-called "fat-sparers" consisting of gelatin, dextrin, or gum tragacanth, have been used to increase the mucilaginous character of the chocolate when dissolved, or its smoothness when tasted, and thus conceal the deficiency of fat. Lecithin, obtained largely from the soy-bean, is also used as an emulsifier to increase the fluidity or smoothness of chocolate "liquors" and permit reduction of fat content, as in chocolate coatings. Very rarely samples have been found in which some mineral adulterant, such as red ochre, has been used to add weight or improve the color, coal-tar dyes being also employed for the latter purpose. It has until recently been a common practice, especially in the summer, to coat the cakes of chocolate with some varnish to prevent their softening in warm weather. Gum benzoin, wax, and even shellac have been used for this purpose, especially with cheap chocolate candies, but are not commonly employed at present, owing to the activity of the Federal authorities.

In the case of *sweet chocolate* or "eating chocolate," which is usually only the plain chocolate with the addition of sugar and flavoring, another form of adulteration is the addition of sugar in excessive amounts. The amount should not ordinarily be above 60 per cent, although quantities as high as even 90 per cent have been found. It should be noted that at the present time the use of dextrose (corn sugar) is allowed on an equality with sucrose.

With *cocoa* the opportunity and the temptation to use various powdered adulterants is even greater. Such adulterants as corn, sago and arrowroot starch, cocoa shells, wood fibers, and ground acorns are readily detected by the microscope. There is, of course, no objection to the sale of cocoa containing foreign starches or sugar provided it is properly labeled. The same cannot be said of cocoa shells, however, which are largely indigestible and are added solely for fraudulent purposes.

The "soluble cocoas" on the market are prepared by heating the beans with steam under pressure or by treating the roasted beans or ground cocoa with some form of alkali, the object being to saponify the fat to a certain extent and render it less

likely to separate in the cup. By the action of the alkali, also, the vegetable tissues are partly disintegrated and the material will remain suspended in water or milk to a greater degree. The cocoa is darkened in color and given the appearance of greater strength. It should, in fairness, be remarked that the process is considered by many to improve the flavor. In the so-called "Dutch process," sodium, potassium, or magnesium carbonate is employed, while in the German method ammonia or ammonium carbonate is the alkali commonly used. Actual determinations of the solubility of a number of the brands for which special claims are made in this regard show that the use of the word "soluble" on the label is based more on fiction than on fact (see page 382).

Sweetened cocoas, as in the case of sweet chocolate, should be examined for the presence of excessive amounts of sugar, the quantity which can be added legally being limited to 60 per cent. Sometimes cocoa, either sweetened or unsweetened, is found erroneously labeled "powdered chocolate," a term that is absurd upon the face of it since chocolate, which without qualification means legally plain chocolate or "cocoa mass," can be prepared only in the form of paste and molded into cakes. Saccharin is occasionally, although not commonly, used in place of sugar in these preparations.

Milk chocolate should be made by adding to the chocolate either condensed milk or milk powder that has been prepared from standard milk containing the full proportion of fat. Such preparations, however, are occasionally made from skimmed milk, or mixtures of starch and powdered cocoa shells are added to cheapen the product. It should be kept clearly in mind that some of the additions listed here under the term "adulteration" cease to become adulterants when their presence is properly declared on the label.

Methods of Analysis

Preparation of Sample.—Cocoa, in the state of a fine powder, requires no preliminary treatment. Chocolate or chocolate preparations in cake form should be carefully shaved with a knife, or may be thoroughly chilled on ice and grated with a nutmeg grater, also chilled. In either case endeavor to take

a representative portion of the cake and handle it with the hands as little as possible.

Moisture.—Two grams of the sample are weighed out and dried in the water oven to constant weight, either directly or after mixing with about 10 grams of ignited sand.

Ash Data.—Use 2 grams of sample and determine the total ash, ash insoluble in water and ash insoluble in acid, exactly as described under General Methods, page 23. Titrate the filtrate from the ash insoluble in water with $0.1N$ hydrochloric acid, as stated on page 25, and report the alkalinity of the soluble ash as cubic centimeters of acid required for the ash of 1 gram of sample. For comparison with older reported analyses, it may be advisable also to determine the alkalinity of the total ash in the same manner, reporting the results in cubic centimeters of $0.1N$ acid as before.

Fat. *a. Lepper-Waterman Method.*[1]—Prepare in a Knorr extraction tube, Fig. 63, a tightly packed mat of asbestos purified as for the determination of crude fiber, page 304, and carefully freed from coarse pieces. Wash this filter with alcohol, ether, and a little petroleum ether. (*All* petroleum ether used in this determination must be redistilled below 60°C.) Weigh 2 to 3 grams of the sample into the tube. Insert the tube into a rubber stopper in a filtering bell jar connected to the suction through a two-way stopcock, taking care that no rubber parti-

Fig. 63.— Knorr extraction tube. (*Courtesy of E. H. Sargent & Co.*)

cles adhere to the tip of the stem. Place a weighed 150-cc. Erlenmeyer flask at such a height that the tube stem passes through the neck into the flask. (The stem of the tube should be lengthened if necessary.) Fill the tube to about two-thirds of its capacity with the redistilled petroleum ether, and by means of a rod having a flattened end stir the sample thoroughly, taking care to crush all lumps. Let stand 1 minute and drain by suction. Regulate the suction so that the collected solvent in the flask will not boil violently. Add the solvent from a wash bottle, at the same time turning the tube between thumb and finger so that the sides of the tube are washed down by each addition. Repeat the extractions, with stirring, until the fat is removed. (Ten extractions will usually be sufficient.) Remove the tube with stopper

[1] *J. Assoc. Off. Agr. Chem.*, **1925**, 705.

from the bell, wash the traces of fat from the end of the stem with petroleum ether, evaporate the solvent, and dry to constant weight at 100°C.

The fat-free sample may be used for the crude-fiber determination.

b. Continuous Extraction Method.—Weigh accurately 2 grams of the sample, and, without previous drying, stratify the charge in an extraction tube, as the Soxhlet or Johnson, with about 0.5·gram of asbestos, or mix with ignited coarse sand. Extract with petroleum ether, boiling below 60°C., in a continuous extractor, preferably of the Johnson type, for 4 hours. Grind the material, to break up any lumps that may have formed, and reextract if desired. It is advisable to allow the solvent to run through the material once completely before applying heat for the continuous extraction. Collect the petroleum ether extract in a weighed flask, evaporate the solvent, and dry the residue to constant weight at 100°C.

The extracted residue in the extraction tube may be used for the determination of crude fiber if purified and ignited asbestos has been used. It is generally preferable, however, to use a separate, larger sample for the crude fiber.

Notes.—The Lepper-Waterman method has been adopted as official by the Association of Official Agricultural Chemists. The continuous extraction, however, which is practically the tentative method of the association, is equally satisfactory and is preferred by many analysts. Both methods differ from earlier ones in the use of light petroleum ether in place of anhydrous ethyl ether, as formerly recommended. The former solvent can be used without previous drying of the sample and does not extract theobromine in addition to the fat, as is the case with ethyl ether.

The material to be extracted by the continuous method should be mixed with asbestos or sand, or possibly wrapped in a filter paper, because of the tendency of cocoa products, being in some cases extremely finely ground, to be carried through with the solvent, and if this be prevented by a dense filtering medium, filtration may be almost stopped before extraction is completed.

With most cocoa products the fat can be determined with sufficient accuracy for ordinary purposes without the reextraction. Experiments in this laboratory have shown that in the

case of cocoa, extraction for 6 hours gave 99.7 per cent and for 8 hours 99.92 per cent, as much ether extract as was obtained by 16 hours extraction including a regrinding of the residue. In any case an extraction for 4 hours, followed by grinding of the residue with an equal quantity of fine sand and reextraction for 2 hours, ought to prove sufficient.

Crude Fiber. *Method.—a. For Cocoa Products Except Milk Chocolate.*—Treat 7 grams of plain chocolate, 50 grams of sweet chocolate or an equivalent amount of cocoa powder, twice with 100 cc. of ether in an 8-oz. nursing bottle, centrifuge, and decant the supernatant liquor each time; dry the residue in an oven at about 100°C. and then powder in the bottle with a flattened glass rod. In some cases it may be found necessary to grind the material in a mortar and extract a third time with ether. Wash in the nursing bottle with three 100-cc. portions of distilled water at room temperature, shaking well each time until no cocoa material adheres to the bottle. Centrifuge after each washing for 10 to 15 minutes and decant the aqueous layer. Wash the residue in the same fashion with two 100-cc. portions of 95 per cent alcohol and one 100-cc. portion of ethyl ether. Transfer the residue to a metal dish, dry to constant weight, and grind in a mortar. Weigh 2 grams of the dried material and determine the crude fiber (D) by the method described on page 305, using linen for both the acid and alkaline filtrations. Calculate the percentage of crude fiber on the moisture-, fat-, and sugar-free basis by multiplying D by 0.7.

Notes.—The value found for crude fiber in cocoa and chocolate products has little meaning unless calculated to the moisture-, fat-, and sugar-free basis, on account of the varying amounts of these substances that may be present. In making this conversion, the factor being about 10, there is a great magnification of any errors in the determination. For example, a sweet chocolate might show 56.0 per cent of sucrose, 35.0 per cent of fat, and 0.50 per cent of crude fiber (as determined on the sample). The sugar- and fat-free cocoa material might conceivably be 7.5 per cent. An error of one unit each in the sugar and fat determinations would cause the cocoa-mass figure to vary from 5.5 to 9.5 per cent and the crude-fiber figures on the sugar- and fat-free basis to vary between 5.34 and 9.40 per cent, depending upon an allowable error in sugar and fat.

For this reason the crude fiber is better determined on a sample weighed from the cocoa mass after removal of the ether-, water-, and alcohol-soluble material and drying. The figure obtained in this way, however, is not strictly comparable to those upon which the accepted standards of purity are based, the difference being due mainly to the water-soluble cocoa material removed. For this reason the result is corrected by an arbitrary factor, which has, however, been found experimentally to be fairly constant.

b. For Milk Chocolate.—Proceed as in the case of sweet chocolate with the following exceptions: Treat the material three times with ether instead of twice, and after drying in the bottle and powdering with the glass rod, shake the residue with 100 cc. of 1 per cent sodium oxalate solution, allow it to stand 30 minutes, centrifuge and decant the supernatant liquid, then continue the washings with distilled water as above.

Notes.—The additional treatment with sodium oxalate in the case of milk chocolate is for the removal of most of the milk protein.

Lockwood[1] has published a valuable paper on the detection of shells in cocoa nibs and powder based on the determination of crude fiber, necessitating, as indicated above, accurate determinations of the latter and has made a critical study of the official method. A strictly standardized procedure has been developed which has been found to give excellent agreement in the hands of different analysts. Important points developed are: The fineness of the fat-free powder is a matter of great importance. It cannot be assumed that the cocoa powder as manufactured is sufficiently fine. The material for the test, defatted if necessary, should be ground by hand to pass No. 10 silk bolting cloth (average openings = 0.145 mm.). It should be freshly dried before weighing, being quite hygroscopic, and a ground-joint assembly, avoiding the use of cork or rubber stoppers when boiling, was found helpful. A further important difference lies in the use of filter paper for both the acid and alkali filtrations, in the latter case a weighed filter being employed. Although this may be somewhat open to question, there can be no doubt that the rapid filtration thus gained is a vital point, the extreme slowness of the filtration through asbestos in a Gooch

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[1] *Analyst,* **1939,** 92–102.

crucible being, as anyone knows who has tried it, often a most objectionable feature and possible source of error. Full details of the process will be found in the paper.

The author finds the average fiber content for cocoa nibs on the moisture- and fat-free basis to be 5.9 per cent (5.5 to 6.3) and for shell to be 18.2 (17.8 to 19.5) per cent. The variations are due to the character and source of the beans and to the extent of the roasting. If the source of the beans and the character of the roast is known, as with factory control, the method seems quite satisfactory. A table is given showing the relation between shell and fiber and numerous figures showing the successful application of the method.

Starch. *a. By Acid Hydrolysis.*[1]—Transfer 4 grams to an 8-oz. nursing bottle or other receptacle which can be used in the centrifuge. Add 50 cc. of gasoline or petroleum ether and shake until the material is completely disintegrated; centrifuge until clear and either decant the gasoline or draw it off by suction. Repeat the process and finally expel the remaining solvent by standing the bottles on the steam bath for a short time. After thus removing the fat, wash the residue, in the case of unsweetened cocoa, into a 250-cc. graduated flask with 200 cc. of hot water and hydrolyze the starch by Sachsse's method as detailed on page 300. Cool the acid solution, nearly neutralize with strong sodium hydroxide, add 5 cc. of basic lead acetate solution, make up to 250 cc., mix thoroughly, and filter through a dry filter. Remove the lead from the filtrate by adding powdered potassium oxalate, avoiding excess, filter through a dry filter, and determine dextrose in 50 cc. of the filtrate by one of the copper-reduction methods, pages 263–275.

With sweetened cocoa products use 10 grams and add to the fat-free residue 5 cc. of alumina cream (page 289) and 100 cc. of water, shake until thoroughly mixed, and centrifuge until the supernatant liquid is clear. Draw this off and twice repeat the shaking and centrifuging with water. Transfer the residue to a 250-cc. graduated flask with 200 cc. of water and proceed as above.

Notes.—The repeated treatment with water is in order to remove sugar, the alumina cream being added to assist in the sedimentation of the fine particles of cocoa.

[1] Dubois: U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 136; *Bull.* **162**, p. 132.

The standard method of the Association of Official Agricultural Chemists requires repeated grinding of the sample under ether to remove the fat. The method will undoubtedly give accurate results if used with care, but is slow and tedious in the extreme. With sweetened samples it often takes 2 days to wash with 500 cc. of water, and in warm weather there is danger of the sample molding during that time. The grinding under ether and transferring back and forth from the filter to the mortar must be done with great care to avoid loss.

b. By Diastase.—Treat 4 grams of the sample with petroleum ether as above. Carefully wash the residue into a beaker with 50 cc. of water and carry out the hydrolysis as described on page 301, except that as cocoa starch is possibly somewhat more resistant to boiling water than other starches, the boiling with water should be for 30 minutes and the digestion with malt extract should be for 2-hour periods.

Note.—The percentage of starch, as determined by acid hydrolysis, is distinctly higher than the result of the diastase method, as would be expected from the considerable amount of nonstarchy carbohydrates present. With cocoa nibs the average values for the actual starch content, as shown by the diastase method, are about 3 per cent lower than by the acid hydrolysis (see Table 50, page 346). For cocoa shells, the difference is even greater, being about 7.5 per cent.

Sugars. Sucrose.—For the determination of sucrose in the presence of dextrose or lactose as in sweet or milk chocolates, the following general method[1] will be found satisfactory.

Place the normal weight (see page 287) of the finely divided material in an 8-oz. nursing bottle, add about 100 cc. of petroleum ether, and shake for 5 minutes to disintegrate the chocolate. Whirl the bottle in a centrifuge until the petroleum ether is clear. Decant carefully, or draw off by suction, and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue near the steam bath or in a warm place until the petroleum ether is expelled. Add exactly 100 cc. of water and shake until the chocolate is loosened from the sides and bottom of the bottle. Loosen the stopper and carefully immerse the bottle for 15 minutes in a water bath kept at 85 to

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[1] DUBOIS: U. S. Dept. Agr., *Bur. Chem. Bull.* **137**, p. 98; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 198.

90°C., shaking occasionally to remove the material from the sides of the bottle. Remove from the bath, cool, and add a measured quantity of basic lead acetate solution to complete precipitation (5 cc. is usually sufficient). Add enough water to make a total volume of 110 cc. of added liquid. Mix thoroughly and filter through a dry filter. Add powdered potassium oxalate to remove the excess of lead, filter again through a dry filter, and polarize in a 200-mm. tube at 20°C. Invert 50 cc. of the filtrate, preferably using the method of inversion in the cold (page 293) and a flask graduated at 50 and 55 cc., and determine the polarization of the acid invert solution at 20°C., multiplying the result by eleven-tenths to correct for the dilution.

Calculation of Results.—Since the total volume of water and lead solution added to the defatted chocolate was 110 cc., the *apparent* percentage of sucrose is found by the formula

$$S = \frac{P - I}{143.2 - \frac{t}{2}} \times \frac{110}{100}. \quad \text{(See page 293.)} \tag{1}$$

Likewise, the *apparent* percentage of lactose (or dextrose) is calculated by the formula

$$L = \left[\left(P \times \frac{110}{100} \right) - S \right] \times \frac{66.5}{52.5}, \tag{2}$$

where P = the direct reading, S = the apparent percentage of sucrose as above, and 66.5 and 52.5 = the specific rotations of sucrose and lactose (or dextrose) respectively (see page 296).

These results for sucrose and lactose (or dextrose) are not exact on account of the expansion in volume of the solution caused by dissolving the sugars. Hence from the combined apparent percentages of sucrose and lactose (or dextrose) calculate the grams of sugar present in the weight of sample taken. Then the true volume $X = 110 + (G \times 0.62)$, where G = weight of total sugar in grams and 0.62 = increase in volume produced by the solution of 1 gram of sugar. Hence the *true* sucrose per cent = $\frac{SX}{110}$ and the *true* lactose (or dextrose) per cent = $\frac{LX}{110}$.

The same method may be used in the case of sweet chocolates which contain sucrose only, but in this case only equation (1) need be used.

The value obtained for lactose or dextrose in this way will be only an approximate value. For more exact results the method of copper reduction should be employed, as outlined on pages 265 and 269.

Notes.—The reason for adding a measured quantity of water rather than making up to the mark in a graduated flask in the usual way is the large amount of insoluble material in cocoa products, which would give an indeterminate volume of sugar solution by the ordinary method.

The employment of an expansion factor is permissible only in case of water-free substances and where no ingredients other than sugars are dissolved. The factor is not a constant for all concentrations, but above 2 grams per 100 cc. the error is so slight as to be negligible.

Equation (1) on page 355 applies to samples that contain sucrose as the only sugar. In the "Official Methods" of the Association of Official Agricultural Chemists will be found a more complicated formula which gives the percentage of sucrose from the direct and invert polarizations apparently equally well in the presence of lactose or dextrose. This formula, however, is in reality only a more exact algebraical expression for the close approximation formulas given here.[1] It is based on an assumed weight of 26 grams and a specific rotation ratio (lactose or dextrose to sucrose) of 0.79. The results are a little higher than with the simple formulas, although the latter are well within the experimental errors.

Keeping the sugar solution at 85 to 90° for 15 minutes before clarifying is to avoid incorrect readings due to possible mutarotation if dextrose or lactose is present.

Lactose in Milk Chocolate.—The lactose can be determined from the polariscopic readings made in determining sucrose, but the result is not exact on account of the relatively small amount of lactose and the influence of any errors in the sucrose determination. Hence the determination by copper reduction is to be preferred.

Procedure.—Determine the reducing sugars before inversion by the Munson and Walker method, as directed on page 267, in 20 cc. of the lead-free filtrate obtained in the sucrose deter-

[1] Private communication from J. Fitelson, U. S. Food and Drug Administration, New York.

mination. Correct for the cuprous oxide due to the sucrose as follows:

Obtain the approximate percentage of lactose from formula (2) on page 355, using the data obtained in the sucrose determination. From the calculated polarimetric sucrose-lactose ratio and the total cuprous oxide obtained as above, determine the amount of cuprous oxide to be subtracted from the total cuprous oxide found, using Fig. 64. Convert the corrected cuprous oxide to milligrams of lactose (L), using the column in Munson and Walker's table marked "Lactose." The percentage of lactose is then obtained from the following relationship:

$$\text{Per cent lactose} = \frac{LX}{10CW},$$

in which X is the true volume found as on page 355, C is the volume of solution in cubic centimeters used in the lactose determination, and W is the grams (normal weight) of sample taken.

Notes.—The Lane-Eynon volumetric method can be used instead of the Munson and Walker if preferred, using suitable corrections for the "sucrose effect."

The weights of lactose can be taken from the column in Munson and Walker's table headed "1 lactose, 12 sucrose," but if the sucrose has been determined the use of the graph, Fig. 64, compiled for varying mixtures from the columns in the table, will give closer results.

On account of the non-sugar material present in the cocoa bean, the so-called "chocolate blank," better results will be obtained if instead of weighing the cuprous oxide directly it be ignited to cupric oxide, or dissolved and the copper determined volumetrically with thiosulphate. The error in weighing as cuprous oxide is not, however, a very serious one.

There is a small amount of reducing sugar present naturally in the cocoa bean, but the quantity is so small that it can be neglected in the determination of added sugars.

Dextrose in Chocolates.[1]—The fact that, under present Federal regulations, corn sugar, dextrose, can be used in sweetened chocolate preparations on a par with sucrose means that it may be necessary to determine dextrose in the presence of sucrose or

[1] FITELSON: *J. Assoc. Off. Agr. Chem.*, **1932**, 618.

of sucrose and lactose. In any case a qualitative test for dextrose is a necessary preliminary step.

Qualitative Test.—To 5 grams of chocolate in a 250-cc. Erlenmeyer flask add 80 cc. of water and boil gently for 5 minutes. Cool, add 10 cc. of Fehling's copper sulphate solution and 6 cc. of 0.5N sodium hydroxide, mix well and filter. Test the filtrate
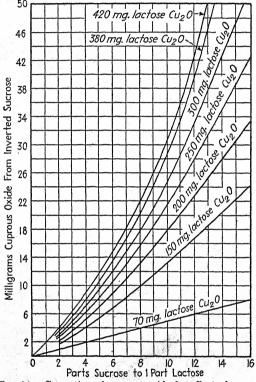

Fig. 64.—Correction of cuprous oxide for effect of sucrose.

by Barfoed's test as described on page 259. One per cent of dextrose can readily be detected.

Dextrose in Sweet Chocolate.—Determine the sucrose by double polarization as on page 293.

Determine by a copper reduction method the total reducing sugars as dextrose in an aliquot of the lead-free filtrate, as described for lactose, page 356. Make a correction for the "sucrose effect," from the ratio of sucrose to dextrose, using

conveniently the Lane-Eynon method with its correction tables (page 265). Since dextrose has the same specific rotation as lactose, the same method of calculation may be used as for lactose.

Lactose and Dextrose in Milk Chocolate. *a. Lactose.*—Defat 10 grams of sample in an 8-oz. nursing bottle as described on page 354 and expel the residual petroleum ether. Transfer the residue quantitatively to a 100-cc. volumetric flask, using about 50 cc. of hot water, cool, and add 2 grams of Fleischmann's compressed yeast that has been stirred into a thick paste with a little water. Rotate the contents of the flask and carefully wash down the sides of the flask with a little water. (The total volume occupied in the flask should be about 60 cc.) Without allowing the contents to splash on the sides, place the flask overnight in an oven kept at about 35°C. or a 37° incubator. Remove from the oven, cool to room temperature, and add 2 cc. of saturated neutral lead acetate solution. Mix, and dilute to 100 cc. Mix, and filter. Delead with dry, powdered potassium oxalate and filter. Determine the lactose in an aliquot of the filtrate by a copper reduction method. If the Munson and Walker method is used, determine the cuprous oxide by thiosulfate titration. (The Lane-Eynon method may be used if the chocolate contains more than 1.4 per cent lactose.) Multiply the results by 0.97 to correct for the volume occupied by the insoluble material. Results will be approximately 0.35 per cent high due to other reducing substances present.

b. Dextrose.—Dextrose in the milk chocolates is best calculated by subtracting the dextrose equivalent of the lactose from the total reducing sugars as dextrose (page 358).

Let L = percentage of lactose determined as above;

f = factor for converting lactose to its dextrose equivalent, based on the copper reduction method used. For the Lane-Eynon method f is 0.738 when 10 cc. of Fehling's solution is used. The equivalent for the Munson and Walker method can be readily ascertained from the appropriate columns, corresponding to the amount of cuprous oxide found.

Then

$$\text{Dextrose} = \frac{DX}{10CW} - fL,$$

where D is the milligrams of total reducing sugars as dextrose in the aliquot taken, corrected for the sucrose effect, and X, C, and W have the same significance as on page 357.

Pentosans.—Follow the method given under General Methods for Carbohydrates, page 303, using 2 to 4 grams of sample.

Total Alkaloids in Cocoa Products.—This determination is useful analytically in showing the presence and approximate amount of chocolate in food products to which it is supposed to have been added, as cake or confectionery.

Method.[1]—Grind 2 to 5 grams of cocoa or chocolate material to a smooth paste with a little alcohol (80 per cent by volume) and transfer to a 200-cc. flask with more of the same alcohol to a volume of about 100 cc. Add 1 gram of freshly ignited magnesium oxide and digest in a boiling water bath for $1\frac{1}{2}$ hours, using an air condenser and shaking occasionally. Filter while hot through a small Buchner funnel, return the residue to the flask and digest again for $\frac{1}{2}$ hour with 50 cc. of the alcohol. Filter and repeat the digestion once more. Evaporate the combined filtrates on the steam bath, adding hot water from time to time to replace the alcohol lost. When all the alcohol is removed finally concentrate to about 100 cc., add 2 to 3 drops of 10 per cent hydrochloric acid, and transfer the liquid to a 200-cc. volumetric flask. Cool, add 5 cc. of zinc acetate solution,[2] mix and add 5 cc. of potassium ferrocyanide solution.[2] Make up to the mark and mix thoroughly. Allow the flask to stand a few minutes and filter through a dry filter paper.

Evaporate a measured quantity (about 175 cc.) of the filtrate to about 10 cc., transfer to a separatory funnel, and extract by vigorous and thorough shaking with five successive 30-cc. portions of chloroform. Wash the combined extracts with 3 to 5 cc. of water. Repeat the process of extraction with five more successive portions of chloroform, wash the second chloroform extracts with the same wash water used before, combine all the extracts, and distill the chloroform. Dissolve the residue in a

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[1] Moir and Hinks: *Analyst,* **1935, 439.**

[2] *Zinc acetate solution.*—Dissolve 21.9 grams of crystallized zinc acetate, $Zn(C_2H_3O_2)_2 \cdot 2H_2O$, and 3 cc. of glacial acetic acid in water and make up to 100 cc.

Potassium ferrocyanide solution.—10.6 grams of crystallized potassium ferrocyanide in water, made up to 100 cc.

little hot water, transfer to a Kjeldahl flask, add 0.2 gram of sucrose and 10 cc. of concentrated sulphuric acid. Heat over a small flame until frothing ceases, add 0.02 gram of selenium (see page 41), digest until colorless and then 1 hour longer. Determine the ammonia by distillation into $0.1N$ acid as on page 42.

Convert nitrogen into alkaloid by the factor 3.26.

Notes.—Alcohol is not necessary for the extraction, water being satisfactory, but the latter is not so well suited if the cocoa material is being determined in the presence of starch or flour, as in cake. Very strong alcohol does not extract the whole of the alkaloids. Magnesium oxide serves as a clarifier and also liberates the alkaloid, which may be present as a glucoside or tannate until set free by the hydrolyzing agent. Loss of alkaloid due to its solubility in the fat separated by removal of the alcohol is prevented by the relatively large volume of hot water. The solubility of theobromine in chloroform is not very great, hence the necessity for prolonged and vigorous shaking and repeated extractions.

The chloroform extract is fairly pure and might be weighed directly in many cases, but for small amounts the nitrogen determination is better. The nitrogen of theobromine is converted to ammonia with considerable difficulty and some danger of volatilization, hence the addition of sugar and the use of selenium as a catalyst.

The nitrogen conversion factor for caffeine is 3.46; that for theobromine is 3.24. The factor used is based on the assumption that 10 per cent of the total alkaloid is caffeine.

For determining cocoa material in cake, confectionery, and similar products, 10 to 20 grams of material should be used. If the amount of mixed alkaloids found is small, the ammonia in the Kjeldahl distillate may be determined colorimetrically with Nessler reagent instead of by titration. In this case a blank must always be run on the reagents. It is usually desirable also to evaporate a portion of the chloroform extract and test qualitatively for cocoa alkaloids by the murexide test, page 345.

Moir and Hinks suggest the average value of 3.15 for the per cent of total alkaloid in moisture- and fat-free cocoa matter for the purpose of calculating the amount of cocoa present in other foods.

Cocoa Red.—Since the cocoa red is found almost entirely in the cocoa bean itself and not in the shells, its determination has been suggested by Ulrich[1] as a method of showing the presence of shells in cocoa.

Place 1 gram of fat-free dry material, which should be finely powdered, in a 300-cc. Erlenmeyer flask, add 120 cc. of acetic acid (50 to 51 per cent strength), connect with a reflux condenser, and boil for 3 hours. Cool and dilute to 150 cc. with cold water; shake well and allow to stand at least 12 hours; filter through a dry filter and treat 135 cc. of the filtrate (corresponding to 0.9 gram of the original substance) with 5 cc. of concentrated hydrochloric acid and 20 cc. of 20 per cent ferric chloride solution. Heat to boiling and boil 10 minutes under a reflux condenser; cool quickly and transfer to a beaker; after standing at least 6 hours, filter upon a weighed filter, washing the precipitate with hot water until free from iron; dry for 6 hours at 105°C. and weigh. Calculate the per cent of the insoluble iron compound in the fat-free sample and thence in the original material.

Notes.—The boiling with dilute acid is necessary to decompose the glucoside, in which form the crude cocoa red is present (see page 345). By hydrolysis the glucoside yields dextrose, theobromine, caffeine, and the true cocoa red, having the formula $C_{17}H_{12}(OH)_{10}$. In its reactions the latter much resembles tannin, forming insoluble compounds with salts of lead, iron, and copper, also with gelatin and albumin.

Ulrich found in determinations on 18 samples, representing eight varieties of cocoa beans, values for the iron precipitate ranging from 5.63 to 7.88 per cent with an average of 6.12 per cent; cocoa shells gave 0.0.

Determination of Milk Proteins in Milk Chocolate.—Weigh 10 grams of the chocolate into a nursing bottle, treat twice with ether, dry and grind in the bottle with a glass rod, as described under Crude Fiber, page 351. Add 200 cc. of 3 per cent sodium oxalate solution and let stand 4 hours, shaking frequently. Centrifuge and filter through a small folded filter. Discard the first 5 to 10 cc. and determine nitrogen in 50 cc. of the filtrate. Pipette 100 cc. of the filtrate into a 200-cc. volumetric flask and dilute almost to the mark. Then precipitate the casein by the addition of 2 cc. of glacial acetic acid. Make up to the mark,

[1] *Arch. Pharm.*, **249**, 524.

shake, filter, and determine the nitrogen in the filtrate as before, using 100 cc. The difference between the two nitrogen determinations gives the nitrogen derived from the casein, which can be multiplied by 6.38 to give the weight of casein present in 2 grams of the chocolate. Casein × 1.25 = milk proteins.

Notes.—The method is based on the greater solubility of casein in hot dilute solutions of the alkali oxalates than is the case with other water-insoluble proteins. The procedure is relatively simple, but the results must be regarded as only approximate since a concentration of the solvent sufficiently great to dissolve all the casein will have some effect on the chocolate protein, as is shown by the fact that distinct values for "casein" are found with some chocolates to which no milk product has been added. Waterman and Lepper[1] have shown that by careful pretreatment of the sample to remove interfering substances and extraction with a solvent buffered at a suitable pH value to give a selective effect, results of higher accuracy can be obtained. See also Winkler[2] for a modified method in which the nitrogen due to casein and albumin is determined directly and somewhat more expeditiously.

From the casein determination the protein content of the added milk may be calculated by multiplying by a suitable factor. The value 1.25 (see page 123) is commonly employed, although it is well recognized that the ratio of casein to other milk proteins is not a strict constant.

Determination of Milk Fat.—Weigh out 5 grams of the dry extracted fat (conveniently obtained from the petroleum ether extract in the determination of sugars, page 354) and determine its Reichert-Meissl number as described on page 230. Assuming 28 as an average value for the Reichert-Meissl number of butter fat and 0.5 as the corresponding value for cocoa butter, the calculation of the amount of butter fat is evident from the following equations, in which X and Y represent, respectively, the grams of butter fat and of cocoa butter present in the 5 grams taken for analysis, and A the Reichert-Meissl value obtained:

$$\frac{28}{5}X + \frac{0.5}{5}Y = A,$$

$$X + Y = 5,$$

[1] *Ind. Eng. Chem.*, **1927**, 501.
[2] *J. Assoc. Off. Agr. Chem.*, **1939**, 603.

By solving for X, knowing the percentage of total fat in the chocolate, the percentage of milk fat is readily determined.

Note.—From the determinations of fat, casein, and lactose, thus made, the approximate percentage of milk solids in the chocolate may be found.

For calculation of the fat in a legal case, however, the Reichert-Meissl value should be taken as 24 rather than 28, the former being the minimum value ordinarily accepted for butter fat.

Examination of the Cocoa Fat.—Separate the fat for examination by extracting a sufficient amount of sample by shaking with two 100-cc. portions of ether, centrifuge, decant and evaporate on the steam bath. Take up the residue in a little ether, filter, evaporate, and dry at 100°C.

For the detection of cocoa-butter substitutes, the determination of the iodine number, saponification number, melting point, and refractive index will often suffice. These may be carried out as described under Edible Fats and Oils, pages 177 to 189. The Reichert-Meissl number may prove helpful, and if coconut oil is suspected, the methods suggested for its detection in butter fat, page 245, will be of service. The determination of the silver number, page 366, is especially advantageous in showing the presence of coconut and palm-kernel oils. Conclusive evidence of the presence of animal fats, as tallow, may be obtained by the phytosteryl acetate test, page 197.

A preliminary test that is often used for the detection of wax or tallow is Björklund's ether test,[1] carried out as follows:

Place about 3 grams of the fat in a test tube, add 6 grams of ether, previously cooled to 18°C., cork the tube, and shake. The fat should dissolve to a clear solution. Then immerse the tube in water at 0°C., and note the number of minutes required for the liquid to become milky, or to deposit white flocks. Note also the temperature at which the solution becomes clear again when removed from the water. The following figures are given by Björklund to show the value of the method:

	Turbidity at 0°C. after minutes	Clear solution at °C.
Cocoa butter	10–15	19–20
Cocoa butter and 5 per cent beef tallow	8	22
Cocoa butter and 10 per cent beef tallow	7	25

[1] *Z. anal. Chem.*, **1864**, 233.

Lewkowitsch[1] recommends that in carrying out this test observation be made also of the characteristic way in which genuine cocoa butter crystallizes as compared with adulterated samples. In the former case tufts of distinct crystals appear on the bottom and sides of the tube while, with 5 per cent or more of tallow, flocks separate from the cooled solution.

Other solubility tests of value are the "critical temperature of dissolution" and the acetone-carbon tetrachloride test. The first of these, practically the same as the Valenta test[2] for fats and oils, is the temperature at which a solution of 5 cc. of a melted neutral fat in 5 cc. of glacial acetic acid becomes turbid on cooling. Practically all potential substitutes for cocoa butter, with the notable exception of hydrogenated oils, tallow, and oleostearin, have a lower temperature of dissolution. Free fatty acids also lower the temperature, and it is necessary to determine the acid value of the fat and make a correction. The temperature of dissolution also varies with the strength of the acetic acid, hence this must be standardized against a sample of cocoa butter of known purity.

The acetone-carbon tetrachloride test is simply the determination of the solubility of the sample in a mixture of equal parts of carbon tetrachloride and acetone. Five cubic centimeters of the dry filtered fat are dissolved in 5 cc. of the carbon tetrachloride-acetone mixture and the solution cooled in ice water for 20 or 30 minutes. If hydrogenated oil, tallow, oleostearin, lard, or paraffin is present, a flocculent precipitate is formed that goes into solution again, but slowly, when the mixture is removed from the ice water and again allowed to come to room temperature.

The critical temperature of dissolution determination is useful in detecting the presence of coconut, palm-nut and cottonseed oils or stearins, corn oil and peanut oil, while the acetone-carbon tetrachloride test will detect hydrogenated cottonseed oil, tallow, oleostearin, and paraffin, but not the other possible adulterants. Detailed directions for making the tests will be found in the "Official Methods" of the Association of Official Agricultural Chemists, **1935,** p. 202.

[1] "Oils, Fats and Waxes," 5th ed., Vol. II, p. 591.
[2] *J. Soc. Chem. Ind.*, **1884,** 643.

Silver Number.[1] *Procedure.*—Weigh 10 grams of fat into a 250-cc. beaker and add 40 cc. of alcohol and 5 cc. of potassium hydroxide solution (750 grams per liter). Saponify the mixture and evaporate to dryness on the steam bath. Take up the soap in 150 cc. of water, warming if necessary. Cool, and make up to 250 cc.

Pipette 200 cc. of the solution into a 500-cc. Erlenmeyer flask. Close the flask with a stopper carrying a thermometer and having a small groove lengthwise in the side. Place the flask in a water bath maintained at about 80°C. When the sample reaches about 80°, loosen the stopper, and introduce 50 cc. of magnesium sulphate solution (150 grams $MgSO_4 \cdot 7H_2O$ per liter) from a pipette. Shake the flask with a rotary motion. Replace the stopper and thermometer and allow the flask to remain in the bath 8 to 10 min. longer at a temperature between 70 and 80°, shaking the flask occasionally. Remove the flask and cool under the tap, with shaking, to 20 to 25°. Remove the stopper and thermometer, stopper tightly, and shake vigorously 4 minutes. Allow the flask to stand in a bath at 20 to 25° until a water layer separates at the bottom. Filter through a Büchner funnel, removing all liquid possible by pressing with a horn spoon. Run a blank on cocoa butter in the same manner.

Neutralize 200 cc. of the filtrate until colorless to phenolphthalein with approximately $0.5N$ sulphuric acid in a 250-cc. volumetric flask. Add 20 grams of sodium nitrate free from chlorides and when dissolved add 22.5 cc. of $0.2N$ silver nitrate solution. Make to the mark and shake 3 minutes. Allow the flask to stand a short time and filter through a folded filter. To 200 cc. of the filtrate add 6 cc. of saturated ferric ammonium sulphate solution and 4 cc. of 40 per cent nitric acid. Titrate with $0.1N$ ammonium thiocyanate to first color change (reddish brown).

Calculate the silver number (milligrams of silver per gram of fat) of the fat by the following formula:

$$\text{Silver number} = (a - b) \times 2.107,$$

in which $a = 1.6 \times$ cc. of $0.2N$ silver nitrate solution added; and $b =$ cc. of $0.1N$ ammonium thiocyanate solution used in back titration.

[1] BERTRAM, Bos and VERHAGEN: *Z. Nahr.-Genussm.*, **1925**, 244; WINKLER: *J. Assoc. Off. Agr. Chem.*, **1934**, 64, 375.

Notes.—The "silver number" is a measure of the insoluble silver salts of the fatty acids after those that form insoluble magnesium soaps have been removed and varies almost directly with the proportion of coconut and palm-nut oils present.

The factor 2.107 is the milli-equivalent of silver times the normality of the $0.1N$ silver nitrate (10.788) divided by the weight of fat (5.12 grams) in the aliquot titrated.

The silver number of palm-nut and coconut oils and stearins varies from about 26 for the stearins to 60 for the entire coconut oil. Dairy butter gives approximately 11.6, and cocoa butter 0.6.

Microscopical Examination.—The principal microscopical characteristics of cocoa are summarized in Chap. II. From the
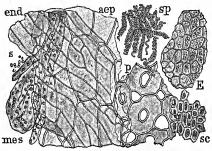
FIG. 65.—Cocoa Shell. Elements in surface view. *sp,* spiral vessels of raphe bundle; *end,* endocarp; *sc,* stone cell layer; *E,* endosperm; *aep,* outer epidermis. × 160. (*A. L. Winton. Reprinted by permission from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

viewpoint of detecting adulterants the most important tissues of the bean are the small, nearly circular starch grains, which exhibit a tendency to gather in groups of two or three; the angular polygonal cells of the cotyledons, which are rather easily broken up; and the masses of yellow, brown, or even violet pigment. These are shown in Fig. 112, page 592.

The most prominent elements of the powdered shell are the spiral vessels, shown in fragments at *b*, and in large masses at *a*, Fig. 113. Typical bundles of spiral vessels (*sp*) and stone cells (*sc*) are shown in Winton's drawing, Fig. 65. Since it is impossible to separate absolutely the shell from the cotyledon in the process of manufacture, occasional fragments of the spiral vessels, which are by far the most numerous distinctive elements of the shell, will be found in genuine powdered cocoa. The occasional occurrence of these should be disregarded, but the

constantly recurring presence of them in large numbers, especially of whole spirals or of bundles of vessels as at *a*, Fig. 113, should be taken as evidence of adulteration with shell powder. Other tissues of the shell, as the epidermal hairs, or "Mitscherlich bodies," and thin epidermal cells, although of interest in studying the structure of the bean, on account of their scarcity or fragile nature are not present in the roasted and ground material in sufficient quantity to be of much analytical importance.

The stone cells and mucilage cells, however, are sufficiently characteristic in appearance and amount to serve as the basis of a quantitative method for the detection of cocoa shells, the only method, in fact, that will show with reasonable certainty the presence of less than 5 per cent of shells. The counting of the stone cells and comparison with standards of known purity is extremely trying and tedious, requiring much perseverance to give good results. In the author's laboratory, however, it has been found a distinct success. The details of the method as worked out by the analysts of the Bureau of Chemistry[1] may be summarized as follows:

Preparation of Standards.—The careful preparation of the standards is of immeasurable importance. It is not advisable to use a commercial cocoa although believed to be free from shell. The only accurate method is to start with clean nibs and shells. Grind and thoroughly defat both nibs and shells separately until each passes through a 100-mesh sieve. Weigh and mix nib powder and shell powder in desired proportions, finally sieving each standard through the 100-mesh sieve to ensure thorough and uniform mixing.

Preparation of Samples.—Extract the fat from a small amount of the sample with gasoline or ether, conveniently with the centrifuge or suction filter. In the case of chocolate, shave up the sample so that the defatting agent will penetrate. Repeat the extraction three or four times, if necessary grinding the sample in a mortar between extractions. In the case of sweetened products remove the sugar by washing several times with water in the same way and wash finally with a mixture of ether and alcohol. Dry, powder, and mix as in the case of the standards. Weigh accurately 2 mg. and mount on a glass slide with just enough of a mixture of chloral hydrate solution (60 per

cent) and glycerol (1:1)[1] to fill in under a square cover glass. Do not use too much of the chloral hydrate-glycerol mixture, since there should be none protruding around the edges of the cover glass. Before applying the cover glass, stir and spread the material with the point of a needle in order to get a uniform mount. Warm slightly (do not boil) and let stand until the tissues have cleared (preferably about 12 hours). The stone cell groups *a*, Fig. 113, page 593, and *sc* in Fig. 65, page 367, are often difficult to recognize and may be confused with other tissues. The only way to gain certainty is by constant study of known samples. Count each group or isolated cell as a unit. Always make a check by counting a standard. A good plan is to count one slide of the sample, then count the standard to which it seems to be nearest in shell content, and then count at least one more slide of the sample. Above all, be systematic in covering the slide and try to be consistent in reporting the stone cells. A mechanical stage and a hand tally or counting device will be found of considerable assistance.

Foreign starches, of which the most common in cocoa are wheat, sago, and arrowroot, are distinguished by their characteristics of shape, size, and markings, as detailed in Chap. II.

Interpretation of Results

Composition of Cocoa Beans and Cocoa Shells.—Extended analyses of shelled cocoa beans (cocoa nibs) and of the shells have been made, following modern methods of analysis, by Winton[2] and by Booth.[3] These are summarized in Tables 51 and 52. Later analyses in the Bureau of Chemistry of 17 varieties of roasted beans and shells, and of cocoa dust or "fines" are summarized in Table 53.

In these analyses, which show in general the maximum variation in composition that may be expected in pure unsweetened chocolate, should be noted especially the ash, ash insoluble in acid and crude fiber, as compared with the corresponding values

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[1] An acidified chloral hydrate mixture is sometimes preferable. It is made by dissolving 45 grams of crystals of chloral hydrate in 25 cc. of dilute (1 + 8) hydrochloric acid and 10 cc. of glycerol.

[2] WINTON, SILVERMAN, and BAILEY: *Conn. Agr. Expt. Sta., Ann. Rept.,* **1902,** 270.

[3] BOOTH, CRIBB, and RICHARDS: *Analyst,* **1904,** 134.

TABLE 51.—ANALYSES OF AUTHENTIC COCOA BEANS AND SHELLS (WINTON)

Determination	Shelled cocoa beans						Shells					
	Air-dry sample			Water- and fat-free sample			Air-dry sample			Water- and fat-free sample		
	Max. per cent.	Min. per cent.	Av.[1] per cent.	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.	Max. per cent.	Min. per cent.	Av. per cent.
Water	3.18	2.29	2.72	......	......	......	6.57	3.71	4.87	......	......	......
Ash (total)	4.15	2.61	3.32	8.81	5.76	7.04	20.72	7.14	10.48	21.97	5.63	11.33
Soluble ash	1.86	0.73	1.16	3.96	1.60	2.46	5.67	2.02	3.67	6.11	2.16	3.97
Ash insoluble in acid (sand)	0.07	0.00	0.02	0.14	0.00	0.05	11.18	0.05	2.51	11.86	0.05	2.70
Alkalinity of ash (cc. 0.1N acid for 1 gm. of sample)	3.35	1.50	2.51	7.12	3.29	5.32	5.92	5.02	5.52	6.47	5.32	5.97
Theobromine	1.32	0.82	1.04	2.92	1.66	2.21	0.90	0.20	0.49	0.97	0.22	0.52
Caffeine	0.73	0.14	0.40	1.55	0.31	0.86	0.28	0.04	0.16	0.31	0.04	0.17
Crude fiber	3.20	2.21	2.64	6.56	4.70	5.61	19.21	12.93	16.63	20.72	13.71	18.01
Starch by acid hydrolysis	12.37	9.30	11.16	25.68	19.80	23.66	13.89	9.87	11.62	15.42	10.47	12.59
Starch by diastase	8.99	6.49	8.07	18.61	13.82	17.10	5.16	3.36	4.14	5.59	3.65	4.47
Total nitrogen	2.54	2.20	2.38	5.41	4.74	5.05	3.17	1.74	2.34	3.41	1.87	2.54
Fat	52.25	48.11	50.12	......	......	......	5.23	1.66	2.77	......	......	......
Constants of fat (ether ext.)												
Melting point, °C.	35.0	32.3	33.3	......	......	......	......	......	......	......	......	......
Refractive index, 40°C.	1.4579	1.4565	1.4573	......	......	......	......	......	......	......	......	......
Iodine number	37.89	33.74	34.97	......	......	......	......	......	......	......	......	......

[1] In each case the average represents 17 varieties.

for shells, and the starch (both by acid and by diastase), which serves to detect such starchy adulterants as wheat flour or arrowroot. Both shells and foreign starches are, of course, more readily detected qualitatively in the microscopical examination, the above determinations aiding in showing the extent of adulteration.

Cocoa shells are much less uniform in composition than the shelled beans, especially as regards the percentages of ash and sand, since they are contaminated by variable amounts of adhering dirt and are more affected by the heat of roasting. An instance is seen in the figures for Puerto Cabello shells in Booth's table:

TABLE 52.—ANALYSES OF NIBS AND SHELLS FROM KNOWN SOURCES (BOOTH)

Nibs	Ash, per cent.	Soluble ash, per cent.	Ash insol. in HCl, per cent.	Alkalinity as K_2O, per cent.	Cold water extract, per cent.	Nitrogen, per cent.	Fat, per cent.	Crude fiber, per cent.
African............	2.52	0.98	0.05	0.38	11.8	1.84	50.2	......
Grenada...........	2.60	1.04	0.03	0.55	9.8	2.26	50.8	2.94
Guayaquil	3.16	1.32	0.04	0.53	11.4	......	......	......
Trinidad...........	2.73	0.95	0.00	0.44	12.0	2.32	55.7	2.48
Caracas...........	3.24	1.58	0.08	0.74	......	......	......	......
Bahia.............	2.68	1.22	0.05	0.51	9.5	1.98	44.4	......
Accra.............	3.22	1.36	0.04	0.41	11.4	2.46	50.6	2.87
Ceylon............	3.81	1.66	0.03	0.67	11.9	2.44	50.2	2.36
Puerto Cabello.....	3.88	1.50	0.13	0.64	12.6	2.35	51.3	3.02
Shells								
Ceylon............	6.61	4.78	1.00	2.54	20.7	2.40	3.1	12.80
African...........	5.63	3.53	1.79	2.63	20.4	2.91	3.5	15.70
Guayaquil	8.19	5.25	1.45	3.36	24.6	2.13	5.9	12.85
Puerto Cabello.....	20.82	5.24	8.33	1.13	23.5	......	5.7	13.83

Calculated to the water- and fat-free basis, which, on account of the great variation of these constituents in different cocoa products, is the only fair basis for comparison, it is seen that the minimum value for crude fiber in the shells is about 14 per cent and the maximum for shelled cocoa is about 7 per cent, from which it is evident that the addition of each 15 per cent of shells increases the crude fiber by at least 1 per cent.

The ratio of crude fiber to total ash is a helpful indication of the addition of shells or fines. In genuine chocolate the quotient

of crude fiber divided by ash is 0.7 to 0.9; in shells and dust it is 1.5 to 2.5. If the crude fiber equals or exceeds the total ash it is evidence of the addition of shells or dust.

It should be noted also that the soluble ash and alkalinity of the ash are both higher in the shells; hence the addition of the latter would increase these values in genuine cocoa, a fact that

TABLE 53.—ANALYSES OF COCOA BEANS, SHELLS, AND DUST (BLOOMBERG)

Moisture- and Fat-free Basis. (Per cent.)

| | Pentosans | Crude fiber | Total | Ash | | | | | Moisture | Fat |
| | | | | Water- | | Acid insoluble | Alkalinity | | | |
				Insoluble	Soluble		Soluble	Insoluble		
Beans										
Maximum..........	4.95	7.67	8.49	5.06	4.11	0.39	3.8	6.9	2.72	56.76
Minimum..........	3.72	5.50	6.22	3.94	2.23	0.00	1.8	5.2	1.42	52.37
Average.........	4.39	6.18	7.77	4.52	3.21	0.22	2.8	6.1	1.98	54.43
Shells										
Maximum..........	11.46	24.37	22.57	19.27	6.51	8.34	7.85	8.5	9.91	11.66
Minimum..........	7.97	14.70	7.21	2.84	2.64	0.09	2.6	3.4	3.76	2.39
Average..........	10.07	18.60	10.42	5.70	4.72	1.57	5.31	4.75	5.77	5.33
Dust (Fat-free Basis)										
Maximum..........	6.27	18.80	22.96	21.40	5.49	12.18	4.20	5.6	.....	43.43
Minimum..........	4.44	9.02	8.45	4.56	1.52	0.49	3.10	5.2	.....	18.00
Average.........	5.49	14.53	13.04	9.45	3.70	3.54	3.80	5.4	.....	27.29

should be taken into consideration in using these values to judge whether a sample has been treated with alkali (see page 381).

Standards for Cocoa Products.—The Federal standards for chocolate and its preparations are as follows:[1]

[1] Service and Regulatory Announcements, U. S. Dept. Agr., *Food and Drug No. 2*, Fifth Revision, November, 1936.

These are the latest official standards available. At the time of writing, however, in conformity with the policy of enforcement of the Food, Drug and Cosmetic Act, a hearing is announced by the Food and Drug Administration of the Federal Security Agency to set up definitions and standards of identity for the various cacao products. Although it would be manifestly impossible to tell just what standards will be finally adopted, it is of interest to note the limits that are proposed for discussion and within which the final standard is to fall.

Briefly, the proportion of shell in *cacao nibs, cocoa nibs,* is to be within the range of 1.0 to 1.75 per cent, as determined by a method strictly specified;

Chocolate, plain chocolate, bitter chocolate, chocolate liquor, chocolate paste, bitter chocolate coating, is the solid or plastic mass obtained by grinding cacao nibs. It contains not less than 50 per cent of cacao fat and, on the moisture- and fat-free basis, not more than 8 per cent of total ash, not more than 0.4 per cent of ash insoluble in hydrochloric acid, and not more than 7 per cent of crude fiber.

Sweet chocolate, sweet chocolate coating, is chocolate mixed with sugar and/or dextrose, with or without the addition of cacao butter, spices, or other flavoring materials. It contains, on the moisture-, sugar-, and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid, or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

Milk chocolate, sweet milk chocolate, is the product obtained by grinding chocolate with sugar and/or dextrose, with the solids of whole milk, or the constituents of milk solids in proportions normal for whole milk, and with or without cacao butter and or flavoring material. It contains not less than 12 per cent of milk solids.

Cocoa, powdered cocoa, is chocolate deprived of a portion of its fat and pulverized. It contains, on the moisture- and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid, or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

"Breakfast cocoa" is cocoa which contains not less than 22 per cent of cacao fat.

Sweet cocoa, sweetened cocoa, is cocoa mixed with sugar and/or dextrose and contains not more than 65 per cent of total sugars in the finished product, and, on the moisture-, sugar-, and fat-free basis, no greater percentage of total ash, ash insoluble in hydrochloric acid or crude fiber, respectively, than is found in moisture- and fat-free chocolate.

Sweet milk cocoa is the product obtained by grinding cocoa with sugar and/or dextrose, with the solids of whole milk, or the constituents of

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chocolate, plain chocolate, etc., is to contain between 50 to 53 per cent and 58 to 61 per cent of cacao fat; *sweet chocolate* is to contain not less than 15 to 20 per cent of the plain chocolate; *cocoa, (medium-fat cocoa)* is to contain not less than 8 to 12 per cent of cacao fat, but less than 22 to 25 per cent, the latter being the minimum standard for fat content of *breakfast cocoa, high-fat cocoa; sweet chocolate* may also contain various optional milk products, as condensed or dried milk, buttermilk, malted milk, etc., such products to contain not less than 5 to 7 per cent of milk solids, but less than 12 to 16 per cent, the latter range being the minimum range for milk solids in *milk chocolate, sweet milk chocolate.*

In general, the addition of optional ingredients, as milk products, lecithin (not more than 0.5 per cent), alkali, spice, seasoning, vanillin, or other flavor to any cacao product must be plainly declared on the label.

milk solids in proportions normal for whole milk, and with or without flavoring material. It contains not less than 12 per cent of milk solids.

Dutch-process chocolate, "*alkalized chocolate,*" and *Dutch-process cocoa,* "*alkalized cocoa,*" are modifications, respectively, of chocolate and cocoa, in that in their manufacture an alkali carbonate, or other suitable alkaline substance, has been employed.

In the preparation of these products not more than 3 parts by weight of potassium carbonate, or the neutralizing equivalent thereof in other alkaline substance, is added to each 100 parts by weight of cacao nibs. The finished products conform to the standards for chocolate and cocoa, respectively, due allowance being made for the kind and amount of alkaline substance added.

The standard for plain chocolate proposed by Whymper[1] at the Berlin Congress of Cocoa and Chocolate Makers in 1911 requires not less than 45 per cent cocoa fat; not more than 4 per cent ash (ratio of soluble to insoluble ash not to exceed 2:3); not more than 6 per cent moisture; not more than 2.75 per cent pentosans; not more than 13 per cent cocoa starch.

Booth[2] has proposed as the result of numerous analyses of milk chocolate that it should be defined as a "preparation composed exclusively of roasted, shelled cocoa beans, sugar, and not less than 15 per cent of the dry solids of full-cream milk, with or without a small quantity of harmless flavoring matter."

Plain Chocolate.—The composition of pure unsweetened chocolate, as sold, naturally resembles closely that of cocoa nibs as tabulated above. There is, however, somewhat less variation in composition, since the product as sold is ordinarily prepared from a blend or mixture of cocoa beans, and is consequently more uniform. This is shown by the following figures for 10 well-known brands examined in 1911.[3]

In Table 55 are given several similar analyses of adulterated plain chocolate.[4] Samples 1 and 2 contained added starch and in sample 3 part of the cocoa butter was replaced by coconut oil. In two of the samples there is a deficiency of fat.

[1] "Cocoa and Chocolate: Their Chemistry and Manufacture," 2d ed., p. 549.

[2] VIIth *Internat. Cong. of Appl. Chem.,* VIIIc, p. 178.

[3] Street: *Conn. Agr. Expt. Sta., Ann. Rept.,* **1911,** 103.

[4] Winton: *Conn. Agr. Expt. Sta., Ann. Rept.* **1903,** 123.

Apart from the presence of foreign starches, the adulterants to be looked for especially in plain chocolate are the addition of shells and of foreign fats. The former is discussed under cocoa powder on page 379. The use of cocoa-butter substitutes is

TABLE 54.—ANALYSES OF PLAIN CHOCOLATE

Determination	Maximum per cent.	Minimum per cent.	Average per cent.
Total ash..........................	3.76	2.91	3.40
Soluble ash.........................	1.66	1.11	1.42
Ash insoluble in acid.................	0.21	0.03	0.10
Alkalinity of ash (cc. 0.1N acid for 1 gram.)............................	4.42	3.29	3.96
Fat................................	52.35	47.05	49.56
Nitrogen...........................	2.46	2.09	2.29
Soluble in cold water.................	14.00	11.02	12.53
Soluble in water at 65°C.............	15.68	12.48	14.04
Soluble in water at 100°C.............	17.64	14.38	16.11
Relative sedimentation...............	60	45	52
Iodine number of fat.................	36.99	34.15	35.32
Refractive index of fat, 40°C..........	1.4572	1.4563	1.4569

TABLE 55.—ANALYSES OF ADULTERATED CHOCOLATE

Determination	1	2	3
Total ash..........................	3.02	2.96	3.96
Soluble ash.........................	1.33	1.25	1.53
Ash insoluble in acid.................	0.07	0.08	0.14
Alkalinity of ash (cc. 0.1N acid for 1 gram.)............................	1.65	1.85	1.95
Fat................................	40.69	46.48	44.61
Nitrogen...........................	2.42	1.99	2.51
Crude starch........................	21.60	19.11	14.00
Pure starch.........................	17.64	15.03	10.53
Crude fiber.........................	2.63	2.42	3.64
Melting point of fat, °C..............	31.75	31.50	29.50
Refractive index of fat at 40°C..........	1.4572	1.4569	1.4548
Iodine number of fat.................	37.83	37.46	26.05

possibly on the increase because of the great demand at present for the smooth, soft chocolate which must be made by finer grinding and by the addition of cocoa butter to the cocoa mass.

The substitutes most commonly employed include the following fats, either alone or in combination: Coconut-oil stearin, palm-

nut stearin, tallow, cottonseed stearin, and shell butter, which is the fat obtained from the shells of the cocoa bean. Vegetable oils, such as peanut and sesame, and even paraffin and beeswax, have been reported. One common substitute sold was found to be a mixture of hydrogenated fat, lanolin, and yellow wax. The analytical constants of most of these have been included in Table 27 on page 199, but for convenience the following values, taken from an extended table by Whymper,[1] are given here.

TABLE 56.—CONSTANTS OF COCOA BUTTER SUBSTITUTES

Fat or oil	Specific gravity at °C.	Melting point, °C.	Saponification value	Reichert-Meissl number	Iodine value
Cocoa butter..........	0.964–0.974 at 15	30.0–34.0	192–195	0.2–0.9	32.0–42.0
Cottonseed stearin.....	0.867 at 100	27.0–45.0	194.5	0.8–1.0	89.0–93.0
Coconut oil...........	0.9259 at 15	20.0–28.0	246–252	6.6–8.4	8.0– 9.0
Coconut stearin....:..	0.8700 at 100	29.3–29.5	252	3.4	4.0– 4.5
Palm-nut oil..........	0.8731 at 99/15.5	23.0–30.0	243–255	5.0–6.8	10.5–17.5
Palm-nut stearin.......	0.8700 at 100	31.5–32.0	242	2.2	8.0
Tallow...............	0.925–0.940 at 15.5	38.0–50.0	193–198	0.2	33–48
Peanut oil............	0.911–0.926 at 15.5	....	186–194	0.5	83.0–101.0
Sesame oil...........	0.921–0.925 at 15.5	....	188–193	1.2	109.0–112.0
Paraffin..............	0.824–0.940 at 15.5	36.7–58.3	0.0	0.0	3.9–4.0

Analyses of authentic samples of cocoa butter and cocoa-butter substitutes, made in the Bureau of Chemistry, are given in Table 57.

The critical temperature of dissolution and acetone-carbon tetrachloride tests are useful preliminary tests. Often if these show the absence of adulterants no further tests need be made. The difference between the melting point and the titer is an important criterion. Adulterants indicated by the preliminary tests may be identified by the determination of the constants.

Such substitutes as coconut and palm-oil stearins will be shown by their high saponification value and low iodine number. In the absence of butter fat, the increased Reichert-Meissl number will also be indicative. The change in melting point may be noticeable, but it is hardly as decisive as the constants mentioned. The specific methods for coconut oil may be employed (see page 245), but would not be of so much value in the case of the coconut stearin. The silver number, page 366 will help here.

[1] "Cocoa and Chocolate: Their Chemistry and Manufacture," 2d ed., p. 478.

The liquid vegetable oils lower the melting point, especially of the fatty acids, and raise the iodine number and refractive index, without affecting to any great degree the saponification value and Reichert-Meissl number. It should be remembered in this connection that cocoa butter obtained from cocoa shells may have iodine values one or two units higher than the ordinary. Paraffin or beeswax, the addition of which is comparatively rare, would be detected by the lowered saponification

TABLE 57.—ANALYSES OF COCOA BUTTER AND SUBSTITUTES

Fat or oil	Density 40°C./40°C.	Refractive index 40°C.	Iodine number	Saponification number	Reichert-Meissl number	Polenske number	Melting point capillary	Titer	M. P.—Titer
Cocoa butter, maximum...	0.9026	1.4575	38.2	194.3	0.40	0.70	35.0	49.8	− 14.6
(7 samples), minimum...	0.9007	1.4572	35.0	193.7	0.20	0.55	34.3	48.3	− 13.3
Shell butters, maximum...	0.9045	1.4620	53.5	193.0	1.25	1.20	34.6	49.0	− 15.7
(6 samples), minimum...	0.9042	1.4582	38.0	182.0	0.00	0.65	33.3	47.8	− 13.8
Cocoa butter substitute...	0.9045	1.4570	32.0	193.9	0.60	0.85	37.8	53.4	− 15.6
Cocoa butter substitute...	....	1.4500	5.0	246.0	5.40	11.50	44.0	27.8	+ 16.2
Cottonseed stearin...	....	1.4630	84.0	202.0	1.00	0.50	43.8	42.7	+ 1.1
Oleo stearin (old)...	0.9109	1.4560	19.5	203.0	1.30	0.80	54.8	51.8	+ 3.0
Beef tallow, edible...	0.9025	1.4575	38.2	201.0	0.20	0.60	47.0		
Coconut and palm-kernel stearin, maximum...	0.9144	1.4495	8.7	256.0	5.20	16.0	33.2	27.0	+ 6.5
(6 samples), minimum...	0.9142	1.4500	6.1	249.0	2.90	10.00	31.4	25.3	+ 4.0
Palm-kernel oil...	0.9158	1.4518	19.0	247.0	6.65	11.40	27.4	22.1	+ 5.3
Coconut olein...	0.9162	1.4495	11.0	257.5	7.00	18.20	26.4	27.1	− 0.7
Hydrogenated cottonseed oil...	0.9022	1.4549	15.6	193.0	0.20	0.30	58.2	57.4	+ 0.8

value and the presence of much unsaponifiable matter. Tallow would raise the melting point and the refractive index while the other constants would remain practically unchanged. Indications of its presence would be given also by Björklund's test or the phytosteryl acetate test.

Sweet Chocolate.—This is, of course, only plain chocolate, to which a certain proportion of sugar and flavoring, usually vanilla, has been added, and is defined as such as in the standards given on page 373. Naturally, the same forms of adulteration that have been considered under plain chocolate would apply here, with the added possibility that excessive amounts of sugar may be present. It should be noted that dextrose, corn sugar, can be used as well as sucrose, and the analyst should be certain that any apparent

abnormality is not due to the partial replacement of one by the other. Although no definite limit is given in the standard for the amount of sugar that may be added, the maximum standard of 60 per cent, adopted for sweetened cocoa, would seem to be a reasonable requirement for sweet chocolate. After correcting for the sugar present, the analysis of sweet chocolate should conform to the figures given for plain chocolate on page 375, and to the standards that have been formulated for the latter.

Typical analyses of sweet chocolates are given in Table 58. The first three are analyses of well-known brands, No. III, a

TABLE 58.—ANALYSES OF SWEET CHOCOLATE

Determination	I	II	III	IV	V	VI
Total ash (per cent)................	1.34	1.26	1.68	1.04	0.88	2.18
Ash soluble in water (per cent)......	0.69	0.92	0.78	0.71	0.53	0.88
Alkalinity of ash, cc. $0.1N$ acid for						
1 gram.........................	1.55	1.51	2.06	1.40	1.22	2.22
Fat (per cent).....................	27.90	31.78	41.19	25.90	27.23	11.22
Nitrogen (per cent).................	0.90	0.63	1.21	1.07	0.89	1.61
Starch (acid hydrolysis) (per cent)...	.....	.....	.....	11.60	9.17	.....
Sucrose (per cent)..................	55.09	53.01	34.55	48.02	56.21	55.53
In fat- and sugar-free material						
Total ash (per cent)................	7.87	8.28	6.93	3.99	5.32	6.86
Nitrogen (per cent).................	5.28	4.14	5.00	4.10	5.46	4.84
Starch (per cent)...................	.....	.....	.....	44.45	55.42	.....
Soluble in cold water (per cent)......	25.45	39.78	27.08	.....	.....	27.22

"Semi-sweet" chocolate, being noticeably lower in sugar content than is usual with sweet chocolate, and for that reason being preferred by some, while IV and V are adulterated samples, the adulterant in each case being foreign starch. The starch in a pure sweet chocolate would ordinarily not exceed 5 per cent, or 10.5 per cent in the sugar-free material. Sample VI, although sold as "powdered chocolate," is not a chocolate at all, but a sweetened cocoa, since a large part of the fat has been removed.

Cocoa.—On account of the removal of a part of the fat, the other constituents will be distinctly higher in the case of powdered cocoa than in chocolate. Calculated, however, to a fat-free basis, they ought to agree with the analyses of roasted cocoa nibs stated on the same basis and given on page 372. The first four

analyses in Table 60 on page 383 are typical of unadulterated powdered cocoa as found on the market.

Detection of Shells.—The most delicate and positive method for detecting shells is by the microscope. This method, however, depends largely upon experience and varies with the skill of the analyst. Moreover, it is largely qualitative and without much study gives little idea of the proportion of shell present, so that help must often be had from the chemical tests. The greatly increased content of ash and crude fiber in shells as compared with the shelled beans has already been noted on page 371 in connection with the analyses tabulated. The consensus of opinion seems to be that the crude fiber is the best chemical method for detecting shells. Even in this case it is doubtful, on account of the variations in different samples of cocoa beans and shell, whether the addition of less than 10 per cent of shells could be declared with certainty. If the general characteristics of the cocoa powder to which addition of shells was suspected were known, the addition of possibly 5 per cent could be detected. Taber[1] suggests an average figure of 19 per cent for fiber in shells for the purpose of calculating the shell content in cocoa. Taking the maximum for shelled beans as 7 per cent, an increase of 1 per cent in fiber above the standard would mean an increase of shell content of approximately 8 per cent. Mixtures of shell and nibs made to test this gave the following results:

Shell present, per cent	Crude fiber found, per cent
2.50	4.60
4.80	7.38
6.00	8.70
6.50	8.85

It is evident from these figures that the detection of small amounts of shells is impossible by the crude-fiber method.

The same conclusion is easily reached mathematically. Taking a mixture of 5 per cent of shells with a fiber content of 18.7 per cent and nibs with a fiber content of 6.5 per cent, the fiber content of the mixture would be 7.1 per cent, which is only slightly above the maximum. With a mixture of 2 per cent of shells with the same fiber content, the resultant mixture would yield a fiber content of 6.8 per cent, which is within the maximum 7 per cent limit.

[1] *J. Assoc. Off. Agr. Chem.*, **1921**, 255.

In some cases, with a closer determination of crude fiber, the matter is not quite so hopeless (see page 352).

Another factor of value, although the determination is tedious, is the estimation of pentosans.   Adan[1] reports from 1.19 per cent to 2.19 per cent of pentosans in cocoa nibs, as against 7.57 per cent to 10.53 per cent in shells; Luhrig and Segin[2] find 2.51 to 4.58 per cent in nibs and 7.59 to 11.23 per cent in the corresponding shells.   Many other observers have reported similar results.

Ulrich, as the result of a critical study of the more important methods for showing the addition of cocoa shells, recommends the estimation of cocoa red by the method described on page 362 as satisfactory, it showing the presence of 10 per cent or more.   Sample VII in Table 60, page 383, is adulterated with cocoa shells.

Winkler[3] has proposed the determination of pectic acid as a means of detecting excessive or added shell.   This is much higher in the shell than in the nib, average results being 0.15 per cent for nibs and 4.8 per cent for shells, on a fat-free, sugar-free basis.   The following table is typical of the results obtained.

TABLE 59.—PECTIC ACID IN PLAIN CHOCOLATE

Sample number	Shell added, fat-free basis, per cent	Pectic acid, fat-free dry basis, per cent
1	0	0.11
2	0	0.14
3	0	0.10
4	0	0.16
5	4.0	0.32
6	9.85	0.66
7	14.2	0.87
8	100.	5.18, 4.92

Details of other results obtained and of the method employed will be found in the original papers.

On account of the high proportion, 50 per cent or more, of shell present in cocoa dust or "fines," the effect of this material in chocolate is shown in the analysis in very similar manner to that of shell itself..   A sample made from 20 per cent dust and

[1] *Bull. soc. chim. Belg.*, **1907**, 211.

[2] *Z. Nahr.-Genussm.*, **1906**, 161.

[3] *J. Assoc. Off. Agr. Chem.*, **1937**, 415; **1938**, 440; **1939**, 600.

80 per cent genuine chocolate was analyzed by Bloomberg in the Bureau of Chemistry with the following results (as per cent on a fat-free basis):

| Pentosans | Fiber | Ash | | | | Fat |
		Total	Water insoluble	Water soluble	Acid insoluble	
5.70	7.73	7.93	4.18	3.67	0.41	49.86

The addition of dust is shown by high pentosans and crude fiber, and high crude fiber-to-ash ratio.

The analysis of three samples, sold as pure chocolate but evidently containing appreciable amounts of cocoa dust, was as follows (on a fat-free basis):

| | Pentosans | Crude fiber | Ash | | | | Fat |
			Total	Water insoluble	Water soluble	Acid insoluble	
1.	4.45	7.31	6.99	3.98	3.01	0.36	52.20
2.	4.75	8.35	7.70	3.75	3.95	0.42	50.56
3.	4.53	8.01	9.59	6.66	2.93	1.18	50.14

The addition of dust is evidenced by high crude fiber in all three, the high crude fiber-to-ash ratio in 1 and 2, high total ash and acid-insoluble ash in 3.

Detection of Added Alkali.—The addition of alkali, in cocoas treated by the so-called "Dutch process," will usually be indicated by the increased proportion of soluble ash and the high alkalinity of the ash, especially of the soluble portion. This is clearly shown in the following comparison of 44 samples of untreated cocoa and 8 samples of alkali-treated cocoa made at the Connecticut Agricultural Experiment Station in 1911. It should be noted that the alkalinity of the ash, as given in the table, refers to the alkalinity of the total ash, not of the soluble portion. This would be distinctly lower, and with untreated cocoa will seldom exceed 3.75 cc. of $0.1N$ acid for 1 gram of sample. In an untreated cocoa or chocolate the soluble ash is

considerably less than the insoluble ash, being usually about one-third of the total ash. It is required under the Federal Food Law[1] that cocoa that has been treated with an alkali or alkaline salt so as to increase the mineral matter present shall bear a label stating the fact that mineral ingredients have been added, and the amount. The amount permissible is distinctly limited (see page 374). Cocoas and chocolates containing an appreciable amount of free alkali are held to be adulterated.

Determination	Untreated cocoa			Alkali-treated cocoa		
	Max. per cent.	Min. per cent.	Average per cent.	Max. per cent.	Min. per cent.	Average per cent.
Total ash...............	6.08	4.33	5.25	9.25	5.96	7.49
Water-soluble ash.......	3.15	1.72	2.32	7.62	4.53	5.79
Alkalinity of ash, cc. 0.1*N* acid for 1 gram...	6.85	4.90	6.04	12.29	7.29	9.36

In Table 60, page 383, analyses V and VI are typical Dutch process cocoas.

"*Soluble*" *Cocoa.*—Although packages of cocoa have been labeled "perfectly soluble" or "pure soluble cocoa," there is in reality no such thing as a "soluble" cocoa. In any case the larger part of the powder is insoluble in water and in those brands that claim to be "soluble," due usually to treatment with alkali, it will be found that, although some of them do not settle as readily from their suspension in water as do untreated cocoas, the difference can be ascribed to the fineness of the powder fully as much as the treatment with alkali. The figures given below, taken from the work of the Connecticut Agricultural Experiment Station, are interesting in this connection.

Determination	Average of 44 straight cocoas per cent.	Average of 8 "soluble" cocoas (alkali-treated) per cent.
Soluble in cold water..................	19.34	19.75
Soluble in water, 65°C.................	21.55	21.33
Soluble in water, 100°C................	23.29	24.99
Organic matter soluble in cold water.....	17.02	13.96
Relative sedimentation.................	96.0	87.0
Ash soluble in cold water..............	44.0	76.0

[1] U. S. Dept. Agr., *Food Inspection Decision* **136.**

These figures show that while 1.7 per cent more of the total cocoa is soluble in boiling water, over 3 per cent less of organic matter is soluble in cold water, *i.e.*, the apparently slightly increased solubility is due to the added amount of alkaline salts and not to any change in the cocoa mass itself. On the whole, the "soluble" cocoas show a lower water-solubility of the *cocoa mass* than do the others. The difference in relative sedimentation is only slight.

In other words, the use of the word "soluble" on cocoa as sold at present is a form of misbranding. To quote again from *Food Inspection Decision* 136: "In the opinion of the Board, cocoa not treated with alkali is not soluble in the ordinary acceptance of the term. Cocoa before and after treatment with alkali shows essentially the same lack of solubility. To designate the alkali-treated cocoa as 'soluble' cocoa is misleading and deceptive."

TABLE 60.—ANALYSES OF TYPICAL COCOA POWDERS

Determination	I per cent.	II per cent.	III per cent.	IV per cent.	V per cent.	VI per cent.	VII per cent.
Total ash.....................	5.07	4.67	4.65	4.52	7.98	6.63	6.84
Ash soluble in water.......	2.02	1.95	1.81	1.76	6.46	5.09	3.32
Alkalinity of ash (cc. 0.1N acid.....................	2.40	2.38	2.20	2.14	5.00	8.32	3.13
Crude fiber...............	3.80	4.26	3.77	3.66	4.39	3.72	10.15
Starch by acid hydrolysis...	15.87	16.57	13.93	14.80	13.71	13.98	8.12
Starch by diastase.........	11.29	12.40	9.16	10.79	9.35	9.16	3.81
Nitrogen.................	3.50	3.27	3.15	3.32	3.33	3.04	2.12
Fat......................	26.22	29.14	34.51	29.55	28.99	29.20	6.82
Theobromine and caffeine..	1.39	1.32	1.21	1.25	1.03	1.28	0.65

The analysis of sweetened cocoa needs no discussion, since apart from the sugar content, which should not exceed 60 per cent, it conforms to the composition of unsweetened cocoa.

Milk Chocolate.—From the ratio between the milk fat and the lactose, determined as described on pages 363 and 356, it can be seen whether the milk used was whole or partially skimmed milk. Comparison may be made with the same ratio calculated from the figures given in Table 13 for the composition of pure milk. From the formulas given on page 138, the approximate percentage of milk solids present may be calculated from the

fat and lactose, or the protein may be calculated directly from the determination of casein, in which case all of the milk solids, with the exception of the ash, are available. The calculation of milk solids from the sum of the lactose, milk fat, casein as determined multiplied by 1.25, and adding 5 per cent of the result for the ash may be preferred to the calculation by the formulas, since in some cases a whole milk is not used in making the milk chocolate, but an extra proportion of milk fat is added.

Analyses of milk chocolate are given by Booth[1] and by Street.[2] Since the latter represent the brands sold on the American market, the typical examples given below are taken largely from that source.

TABLE 61.—TYPICAL ANALYSES OF MILK CHOCOLATE

Determination	I, per cent.	II, per cent.	III, per cent.	IV, per cent.	V, per cent.	VI, per cent.
Total ash......................	1.56	1.85	1.71	1.67	1.79	1.16
Ash soluble in water............	0.54	0.63	0.66	0.85	0.82	0.40
Alkalinity of ash, cc. 0.1N acid for 1 gram......................	1.19	1.82	2.09	1.93	2.10	0.86
Nitrogen......................	1.17	1.36	1.19	1.11	1.42	......
Sucrose.......................	48.31	45.81	43.09	49.45	39.45	48.88
Lactose.......................	7.28	7.75	3.57	2.25	6.24	1.12
Fat...........................	29.95	28.69	32.13	28.77	33.23	37.02
Reichert-Meissl number of fat.....	6.2	5.9	5.0	3.2	4.1	8.8
Butter fat (calculated)...........	7.72	7.06	6.68	3.82	5.68	13.09

It is clearly shown by inspection of the values for lactose and fat in the table that in every case milk distinctly above the average in quality was employed. Sample VI, which was an imported chocolate, analyzed in the author's laboratory, was labeled "Cream Chocolate," and the analysis confirms this. It is hardly necessary to point out that neither the lactose nor the butter fat alone is evidence of the character of the milk used, but the ratio between the two must be considered. Sample V shows a higher content of butter fat than does sample IV, but the milk used was of inferior quality. The actual amount of milk solids present should, of course, be taken into account also.

[1] *Analyst*, **1909**, 146.
[2] *Conn. Agr. Expt. Sta., Ann. Rept.*, **1911**, 106.

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CHAPTER VIII

SPICES

Spices are of especial interest to the student of food analysis, not only because they afford perhaps the best opportunity for the happy combination of chemical and microscopical tests, but also because from their nature they are far more likely to be adulterated than would be imagined from their actual monetary value.

The microscopical examination, as explained more fully in Chap. II, is of the greatest value in pointing out quickly and qualitatively the character of the adulterants present, while the chemical tests may serve to confirm the indications of the microscope as well as to show some forms of adulteration, such as partially exhausted or improperly cleaned spices, which are not revealed by the microscope.

The general adulterations of spices consist largely in the addition of such inert, refuse materials as ground nutshells, sawdust, bark, fruit stones, and roasted cereals. Cheaper spices may be substituted in part for the more costly ones, as allspice for cloves, or small quantities of the more pungent spices, as cayenne, may be employed to conceal by their stronger taste the addition of diluents, as starches. Such adulterations as the substitution of inferior grades of the same spice, the removal of a portion of the volatile oil by steam distillation, or the presence of an excessive amount of sand and dirt, either adhering to the spice through lack of thorough cleaning or added with fraudulent intent, are shown best by chemical methods.

Certain general methods, applicable to all of the spices, will first be described, followed by a more detailed discussion of several of the more important individual spices.

GENERAL ANALYTICAL METHODS

Often the sample of spice will be sufficiently fine to examine without further preparation. Any that show an excessive

amount of coarse particles should be ground until they will pass a sieve having round holes 1 mm. in diameter or one having 60 meshes to the inch. In all cases mix the sample thoroughly when taking a portion for examination to correct for lack of uniformity and tendency to form layers on standing.

Moisture.—Weigh 2 grams of the sample and dry it in an air bath at 110°C. to constant weight, which usually requires about 12 hours. From the total loss in weight deduct the percentage of volatile ether extract, determined as described below; the difference is the moisture.

Note.—The above method has been adopted as a provisional method by the Association of Official Agricultural Chemists, but it should be noted that with spices containing a considerable proportion of volatile oil the method is not exact.

Ash and Alkalinity of Ash.—Use 2 grams and determine the total, water-soluble and acid-insoluble ash as described under General Methods, page 23.

Ether Extract. *Total Extract.*—Weigh out 2 grams of the sample and extract it for 20 hours with anhydrous ethyl ether. Some form of continuous extraction apparatus, such as the Soxhlet or Johnson, should be used. For the reasons given on page 35, the latter form of apparatus will be found preferable. Note carefully the precautions there given in the description of the method.

After the extraction allow the ether to evaporate spontaneously at room temperature, then place the flask or dish in a desiccator over fresh, concentrated sulphuric acid for 18 hours, and weigh.

Non-volatile Extract.—Heat the flask containing the weighed ether extract very gradually to 100°C., taking several hours to reach that temperature. Finally heat at 110°C. to constant weight. The residue is the non-volatile ether extract.

Volatile Extract.—This is the difference between the total and the non-volatile ether extract just determined.

Notes.—The precautions as to the rate and temperature of heating are to avoid loss of volatile oil by oxidation or volatilization at too high a temperature.

The determination of ether extract is, on the whole, the most important in determining the purity or value of a spice, since in nearly every case the pungency or essential quality of the spice

is due to ingredients soluble in ether. This may be mainly a volatile oil, as in cloves, in which case the volatile ether extract would be of most importance, or to some non-volatile substance, like the *capsaicin* of cayenne, which would appear in the residual portion of the ether extract. If the essential oil makes up a considerable proportion of the spice or it is desired to make further tests on the oil, the method described under Cloves, page 409, may be used.

Crude Fiber.—Wash 2 grams of the sample on a filter with several portions of ordinary ether or use the extracted residue from the determination of ether extract. In either case proceed as directed on page 304. Previous to weighing the crude fiber treat the crucible several times with ether in order to be certain that all ether-soluble material has been removed.

Notes.—The determination of crude fiber is of importance, in examining spices, since the adulteration consists frequently in the addition of waste or refuse material derived from the spices themselves or other food products. This material is frequently the outer cellular layer or protective coating of the plant, designed to protect the softer tissues that constitute the edible portion, and as such contains more of the hardened or lignified cells, which means higher values for the crude fiber. This is apparent from the following tabulation of the approximate values for crude fiber in some common spices and adulterants:

Material	Per cent of crude fiber
Cloves	7–9
Mustard	2.5–3.5
Mustard hulls	10–20
Pepper	10–18
Pepper shells	25–30
Nutshells	50–55
Sawdust	50–60
Olive stones	55–60
Buckwheat hulls	45

It is worth noting in this connection that the residue on the Gooch crucible in the crude fiber determination is excellent material in which to find stone cells or other hard tissues for the microscopical examination. A bit can be taken out from the moist residue on the tip of a knife and transferred to a slide for examination without interfering appreciably with the determination.

Starch.—The method to be followed depends upon the character of the material and the accuracy desired. If the starch content is high and there is comparatively little material which would yield copper-reducing substances upon hydrolysis, the method by direct acid hydrolysis will be fairly satisfactory. In the case of such spices as white pepper and ginger this method could be used with sufficiently good results in many cases.

On the other hand, with such spices as cloves and mustard, where there is practically no starch, but a relatively large percentage of other copper-reducing substances, either directly or after acid hydrolysis, erroneous conclusions may be drawn unless the diastase method be employed.

It may be said, however, that on account of the greater ease and rapidity of the direct conversion method it is generally employed, due consideration being given to the high results found with some of the spices and the presence of foreign starch reported only when confirmed by microscopical examination. In doubtful cases, or if legal action may result from the conclusions of the analyst, the diastase method should be employed.

In any case, use about 4 grams of the sample, wash with ether, then with alcohol, and proceed as directed on page 299, *et seq.*

Pepper

Black pepper is the dried fruit of *Piper nigrum*, a perennial woody vine indigenous to the forests of India, but now extensively cultivated throughout the East Indies. The small fruit grows loosely on pendulous spikes, of which 20 to 30, each containing 20 to 40 berries, are produced by a single vine. The pepper berry is a small, round, sessile, fleshy fruit, which first appears green, then red, and finally yellow when ripe.

The berries are gathered when beginning to turn red, partially dried on the ground or on mats during several days, then rubbed from the stalks with the hands. When the berries have become thoroughly dried they are shriveled and turn very dark brown or black.

White pepper is not the product of a separate plant, but is the ripened fruit of the black pepper vine, the changed appearance being due to its method of preparation. The berries are soaked in water or buried in damp soil, and after some days

will swell and burst the outer husk, which is then easily removed by rubbing with the hands while the berries are drying in the sunshine, leaving the inner white portion of the berry. Another way is said to be by placing the black pepper in a solution of chloride of lime to remove the dark coating, after which it is rubbed and dried as above.

There are in all about forty different species of the pepper plant, but not all are of commercial importance. The varieties that enter into commerce are usually named from the city or country of export, as Tellicherry, Malabar, Penang, and Singapore. Acheen and Lampong pepper comes from the west and east coasts of Sumatra, respectively.

Shot pepper is one of the heavier grades of black pepper put through a soaking and hardening process that gives it a better appearance and brings for it a higher price.

General Composition.—Pepper contains, in addition to starch, which is present in considerable amount, a small quantity of volatile oil, two characteristic alkaloids, *piperin* and *piperidin*, and slight percentages of gum and resin.

The pungency of pepper is due probably in great part to the alkaloid and the resin, modified to a certain extent by the volatile oil.

Analyses of Genuine Pepper.—Many analyses of authentic samples of pepper will be found in the literature. Some of them are obviously incomplete, and many of the older ones, being made by inexact methods, possess only historical value. For this reason it seems best to give here instead of a general summary of the analyses, thus including those of doubtful value, only two series (see Table 62) made by modern methods and representing the commercial varieties found on the American market.

Another excellent series of analyses has been made by Doolittle,[1] comprising 45 samples of black pepper, representing 12 varieties, and 25 samples of white pepper, including 9 varieties. These are summarized in Table 63.

Finally, in Tables 64 and 65 are a number of analyses of pepper of more recent date, representing the grades imported to this country, the greater portion of which is at present probably Lampong pepper.

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[1] *Mich. Dairy Food Comm., Bull.* **94**; Leach-Winton: "Food Inspection and Analysis," 4th ed., p. 446.

TABLE 62.—ANALYSES OF GENUINE PEPPER[1]

Determination	Black pepper			White pepper		
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.
Moisture...............	12.95	10.63	11.86	14.47	12.72	13.47
Total ash..............	6.85	3.09	5.10	2.96	1.03	1.77
Ash soluble in water...........	3.20	1.75	2.60	0.80	0.28	0.47
Ash insoluble in acid..........	1.63	0.00	0.70	0.20	0.00	0.10
Volatile ether extract.........	2.20	0.65	1.28	0.95	0.49	0.63
Non-volatile ether extract.......	10.37	6.86	8.41	7.94	6.26	6.91
Alcohol extract...............	11.86	8.31	9.44	8.55	7.19	7.66
Copper reducing matters by acid conversion................	43.47	28.15	38.28	64.92	56.43	59.17
Starch by diastase.............	39.66	22.05	33.28	63.60	53.11	56.47
Crude fiber	18.25	10.75	13.62	4.25	0.54	3.14
Albuminoids.................	13.81	10.50	11.93	11.19	10.44	10.89
Total nitrogen................	2.53	2.03	2.25	2.13	1.95	2.04
Total nitrogen in non-volatile ether extract................	0.39	0.27	0.33	0.34	0.26	0.30

[1] WINTON, OGDEN, and MITCHELL: *Conn. Agr. Exp. Sta., Ann. Rept.*, **1898**, 198.

TABLE 63.—ANALYSES OF UNADULTERATED PEPPER

Determination	Black pepper			White pepper		
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.
Moisture...............	11.96	8.09	9.54	13.34	8.04	9.87
Total ash..............	7.00	3.43	4.99	4.28	0.86	1.69
Ash soluble in water...........	3.32	1.65	2.49	1.16	0.12	0.34
Ash insoluble in acid..........	1.80	0.05	0.58	0.86	0.05	0.19
Starch by diastase.............	41.75	25.09	36.69	63.55	48.88	54.37
Volatile ether extract.........	2.10	0.85	1.30	1.66	0.78	1.17
Non-volatile ether extract	10.44	6.60	7.67	7.26	5.65	6.46
Crude fiber..................	18.89	10.05	11.12	7.65	0.10	4.17
Total nitrogen................	2.38	1.86	2.11	2.14	1.85	1.97
Nitrogen in non-volatile ether extract................	0.45	0.25	0.31	0.34	0.24	0.30
Albuminoids.................	13.12	9.25	11.20	11.56	9.69	10.44

In Table 66 are given for comparison similar analyses compiled from various sources, of common adulterants of pepper.

TABLE 64.—RESULTS OF ANALYSIS OF AUTHENTIC SAMPLES

Sample number	Moisture, per cent	Non-volatile ether extract, per cent	Crude fiber, per cent	d-Glucose,[†] per cent	Alcohol extract, per cent	Nitrogen, per cent
						Lampong
Maximum........	12.38	10.74	13.70	51.32	12.26	2.28
Minimum........	8.73	7.30	11.76	44.28	10.22	1.84
Average..........	10.06	9.29	12.50	47.20	11.20	2.09
						Aleppi
Maximum........	10.86	10.46	13.02	51.76	14.34	2.22
Minimum........	9.80	7.97	10.85	47.48	10.00	1.95
Average..........	10.13	8.95	11.66	50.36	11.15	2.08
						Tellicherry
Maximum........	10.27	9.37	14.36	53.36	11.20	2.16
Minimum........	8.20	7.25	11.56	51.20	9.60	1.97
Average..........	9.34	8.31	13.08	51.90	10.35	2.06
						Singapore
33	11.23	9.50	13.83	49.00	9.54	2.28
34	11.00	9.35	13.92	49.04	9.94	2.15
					White and Decorticated	
Maximum........	11.65	9.70	4.86	76.56	9.36	2.02
Minimum........	10.34	6.18	1.03	64.88	7.38	1.56
Average..........	11.05	7.79	3.64	68.64	8.50	1.89
						Shells
39	8.92	9.54	28.52	19.08	9.02	2.07
40‡	11.42	8.87	21.65	25.92	11.44	2.09
41‡	11.10	9.33	22.46	26.44	11.74	2.21
42	9.16	6.40	27.22	19.20	8.18	2.20
						Siftings
43	7.07	4.20	17.04	11.92	5.82	2.47
44	6.22	3.93	19.49	13.84	5.50	1.79
45	8.08	5.09	22.58	20.68	6.24	2.14

* SMITH, ALFEND, and MITCHELL: *J. Assoc. Off. Agr. Chem.*, **1926**, 333.

† Direct acid hydrolysis of 2.5-gram sample without any previous treatment; hence a very crude measure of the starch content rather than actual reducing sugar. In the "Official Methods" it is directed to be reported as "starch."

‡ These two samples of shells evidently contain some endosperm and are not so representative of true pepper shells as numbers 39 and 42.

Forms of Adulteration.—Partly because of the extent to which pepper is used, it being the spice most generally added to foods, and partly because its appearance to the eye is not uniform but

that of a mixture of particles of different colors, the adulterants of pepper are perhaps more varied than with any other spice.

OF WHOLE PEPPER, PEPPER SHELLS, AND PEPPER SIFTINGS*

Total, per cent	Ash Water- Soluble, per cent	Ash Water- Insoluble, per cent	Acid-insoluble, per cent	Alkalinity of ash cc. 0.1 N acid per 1 gram Soluble	Alkalinity of ash cc. 0.1 N acid per 1 gram Insoluble	CaO, per cent	MgO, per cent	P₂O₅, per cent
(18 samples)								
6.29	2.97	3.88	1.02	2.9	4.8	0.89	0.40	C.50
4.39	1.98	1.99	0.11	2.3	2.5	0.61	0.37	0.38
5.05	2.33	2.72	0.41	2.6	4.1	0.69	0.38	0.41
(9 samples)								
5.83	3.61	2.66	0.36	3.0	3.9	0.67	0.39	0.46
4.18	2.50	1.70	0.02	2.4	2.3	0.53	0.34	0.40
4.74	2.88	1.86	0.11	2.6	3.3	0.60	0.37	0.44
(5 samples)								
4.75	2.87	1.99	0.11	2.6	3.5	0.61	0.37	0.47
4.41	2.54	1.71	0.07	2.4	2.4	0.54	0.35	0.42
4.55	2.71	1.84	0.08	2.5	3.0	0.56	0.36	0.45
(2 samples)								
4.17	2.32	1.85	0.16	2.4	3.4	0.59	0.38	0.45
4.06	2.12	1.94	0.25	2.3	3.2	....	0.38	0.46
(4 samples)								
4.84	0.50	4.34	1.28	0.3	2.2	0.37	0.21	0.44
0.83	0.07	0.76	0.05	0.1	0.9	0.23	0.10	0.37
2.01	0.23	1.78	0.37	0.2	1.5	0.31	0.14	0.40
(4 samples)								
10.84	4.09	6.75	2.40	4.8	7.9	1.09	0.72	0.38
7.19	4.49	2.70	0.30	5.1	5.3	0.87	0.57	0.40
7.32	4.42	2.90	0.30	5.2	5.5	0.89	0.59	0.31
10.89	3.46	7.43	2.22	4.1	7.6	1.08	0.72	0.33
(3 samples)								
37.16	0.88	36.28	20.45	0.7	...	0.37	....	0.37
32.21	0.73	31.48	16.50	0.4	...	1.03	....	0.36
20.96	1.46	19.50	9.92	0.7	...	1.09	0.56	0.40

The kinds of refuse and inert material that are added to pepper have been mentioned already, and a fairly complete list of substances that have been reported at various times is given on page 396. The most common of these are probably buckwheat middlings and ground olive stones, in addition to such substances as pepper shells and long pepper, which may be regarded as characteristic adulterants of pepper.

An inferior or low-grade pepper, such as classes C and D of Acheen pepper, containing a large proportion of light berries

TABLE 65.—PORTION OF RESULTS IN TABLE 64 COMPUTED TO A DRY BASIS

Sample number	On dry basis			Ratios	
	Crude fiber, per cent	d-Glucose, per cent	MgO, per cent	MgO × crude fiber	MgO × 1,000 / d-glucose
Lampong (18 samples)					
Maximum............	15.3	57.2	0.44	6.4	8.9
Minimum............	13.1	49.5	0.41	5.2	7.4
Average.............	13.9	52.5	0.42	5.8	8.1
Aleppi (9 samples)					
Maximum............	14.5	57.9	0.43	6.2	7.9
Minimum.......... ...	12.1	52.4	0.38	4.6	6.7
Average.............	13.0	56.1	0.41	5.3	7.3
Tellicherry (5 samples)					
Maximum............	15.8	59.2	0.41	6.2	7.2
Minimum............	12.9	56.6	0.39	5.3	6.8
Average.............	14.4	57.5	0.40	5.8	7.0
Singapore (2 samples)					
33	15.6	55.2	0.43	6.7	7.8
34	15.6	55.1	0.43	6.7	7.8
White and Decorticated (4 samples)					
Maximum............	5.5	85.4	0.24	1.2	3.3
Minimum............	1.2	73.2	0.11	0.1	1.4
Average.............	4.1	77.9	0.16	0.7	2.1
Shells (4 samples)					
39	31.6	21.2	0.80	25.3	37.8
40	24.4	29.3	0.64	15.6	21.9
41	25.3	29.7	0.64	16.2	21.5
42	30.0	21.1	0.79	23.7	37.4
Siftings (3 samples)					
43	18.3	12.8			
44	20.8	14.8			
45	24.6	22.5	0.61	15.0	27.1

and empty shells, may be substituted in part for the high-grade Penang or Singapore spice, especially when sold under a definite trade name. To conceal the addition of diluents such as cereals or olive stones, which would detract from the flavor of the mixture, small quantities of pungent spices as cayenne or mustard

TABLE 66.—ANALYSES OF PEPPER ADULTERANTS

Determination	Coconut shells	Spruce sawdust	Oak sawdust	Pepper shells	Long pepper	Linseed meal	Olive stones	Cocoa shells	Buckwheat hulls	Exhausted cubebs	Exhausted allspice
Moisture, per cent	7.36	8.77	5.73	11.0– 7.0	10.1– 8.4	8.71	9.50	10.44	7.63	5.60	7.69
Total ash, per cent	0.54	0.23	1.22	28.8– 7.8	14.4– 6.1	5.72	0.88	8.40	1.84	10.38	4.50
Ash soluble in water, per cent	0.50	0.16	0.32	4.7– 1.5	4.4– 1.7	1.74	0.24	4.66	1.24	6.32	2.59
Ash insoluble in acid, per cent	0.00	0.00	0.20	22.9– 0.8	5.9– 0.45	0.55	0.44	0.83	0.00	0.77	0.00
Volatile ether extract, per cent	0.00	0.07	0.07	1.1– 0.9	1.0– 0.8	0.04	0.06	1.00	0.07	1.32	0.42
Non-volatile ether extract, per cent	0.25	0.77	0.84	4.7– 1.5	7.5– 5.7	6.58	0.24	2.99	0.38	8.58	6.07
Copper-reducing matter by acid conversion, per cent	20.88	15.48	17.10	....	....	21.15	....	8.68	20.51	....	....
Starch by diastase, per cent	0.23	1.13	1.68	11.7– 9.3	45.8–28.4	14.06	1.73	3.15	1.46	8.55	7.42
Crude fiber, per cent	56.19	64.03	48.79	22.2–21.0	10.0– 7.2	8.30	57.46	14.12	43.76	27.64	22.89
Total nitrogen, per cent	1.13	0.56	1.63	1.8– 1.7	2.1– 2.0	5.09	0.17	2.59	0.17	1.80	1.03

hulls may be added, and the color may be helped out by such substances as turmeric, charcoal, or even coal-tar dyes.

Methods of Analysis

The methods for determining the ash data, the ether extract, starch, and crude fiber have already been described or referred to under the General Methods for examination of spices, pages 387 to 389.

Nitrogen.—Pepper is one of the food substances in which the Kjeldahl or Gunning method for nitrogen, as ordinarily used, does not give correct values. This is due to the piperin, which was shown a number of years ago by Arnold and Wedemeyer[1] to be incompletely decomposed. The modification described on page 38, however, will give perfectly satisfactory results, using about 1 gram of the sample and carrying out the test exactly as described.

Note.—The determination of nitrogen made as described will include both that present in the form of piperin or other alkaloid, and that present in protein form. The latter may be determined, if desired, by subtracting from the total nitrogen the amount of alkaloidal nitrogen determined separately. The results in the line marked "Albuminoids" in Table 62 were obtained in this way, multiplying the difference in nitrogen content by 6.25.

More commonly the nitrogen is determined on the non-volatile ether extract.

Microscopical Examination.—A synopsis of the microscopical characteristics of pepper and of its chief adulterants will be found in the chapter on the Microscopical Examination of Foods, page 62. The principal adulterants shown by the microscope are excess of pepper shells, nutshells (coconut, walnut, almond, etc.), long pepper, olive stones, mustard hulls, cayenne, buckwheat, and other cereals, peas and other leguminous seeds, allspice, turmeric, sawdust—in fact any waste material that can be easily reduced to a powder.

Characteristics of Pepper.—The larger works on the microscopy of foods, as Winton,[2] Schimper,[3] or Tschirch and Oesterle[4]

[1] *Z. anal. Chem.*, **1892**, 525.
[2] "Microscopy of Vegetable Foods."
[3] "Mikroskopische Untersuchungen der Nahrungs.- und Genussmittel."
[4] "Anatomischer Atlas der Pharmakognosie."

illustrate in great detail the structural elements to be found in pepper. Figure 66, from Winton, shows a great number of elements which may be found in the powdered sample. Many of those tissues, however, are of little analytical importance since they occur but seldom or are so easily disintegrated as to be recognized only by careful study. Those of greatest importance are the masses (*am*) of the minute starch grains, the stone cells (*ast*), the cup-shaped "beaker cells" (*ist*) and the occasional bits
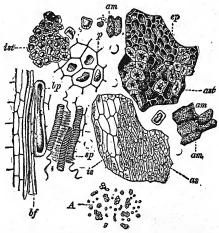
FIG. 66.—Black Pepper. Elements of powder. *ep*, epicarp; *ast*, hypodermal stone cells; *sp*, vessels; *est*, endocarp; *am*, starch masses. × 120. *A*, starch grains. × 450. (*Moeller*.)

of dark-brown polygonal cells of the epicarp (*ep*). Crystals of piperin may also show under the microscope (Fig. 126, page 595).

White pepper shows hardly anything but the starch, both in masses and as individual grains.

Photomicrographs of some of these tissues are shown in Figs. 124 and 125, pages 594 and 595.

Adulterants.—Practically all of the list of adulterants given above will be found mentioned either under pepper or among the other spices described on pages 58 to 63, the essential microscopic structures best suited for their identification being noted in each case. It will bear repeating here that the only successful way for the beginner to identify the adulterants is to proceed systematically, one step at a time, as stated on page 58, and to examine known samples of pepper and of the adulterants until

he becomes familiar with the structures described. A working knowledge of the microscopy of pepper is not difficult to acquire and is of great value in supplementing the chemical analysis.

Color Tests.—Various color tests have been proposed to show adulterants in pepper, especially for ground olive stones and long pepper. A discussion of some of the best of these may be found in Leach-Winton[1] and Parry.[2] In the writer's experience, however, they are less delicate and less convenient than the microscopical examination.

INTERPRETATION OF RESULTS

The Federal standards[3] for pepper are:

Black pepper is the dried immature berry of *Piper nigrum* L. It contains not less than 6.75 per cent of non-volatile ether extract, not less than 30 per cent of starch, not more than 7 per cent of total ash, nor more than 1.5 per cent of ash insoluble in hydrochloric acid.

Ground black pepper is the product made by grinding the entire berry of *Piper nigrum* L. It contains the several parts of the berry in their normal proportions.

White pepper is the dried mature berry of *Piper nigrum* L., from which the outer coating (or the outer and inner coatings) have been removed. It contains not less than 7 per cent of non-volatile ether extract, not less than 52 per cent of starch, not more than 5 per cent of crude fiber, not more than 3.5 per cent of total ash, nor more than 0.3 per cent of ash insoluble in hydrochloric acid.

By comparison with the tables of analyses of genuine pepper previously given it will be seen that these standards are reasonably liberal, and there should be no difficulty in marketing a pepper that will conform to them.

It is seldom necessary in making a chemical analysis of the sample to complete all the tests given in the tables. The requirements laid down in the standards cover the ground quite fully, and for many purposes the determinations of non-volatile ether extract, of its nitrogen content, and of crude fiber furnish sufficient information. When black pepper is adulterated with

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[1] "Food Inspection and Analysis," 4th ed., p. 450.

[2] "Food and Drugs," Vol. I, p. 206.

[3] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

such materials as buckwheat hulls, charred coconut hulls, or other substances low in ether extract, the amount of non-volatile ether extract will be diminished without sensibly decreasing the proportion of nitrogen in 100 parts of the extract. When, on the other hand, linseed meal, or other oily material has been added, the percentage of non-volatile ether extract may remain about the same as in genuine pepper, but the parts of nitrogen in 100 of the extract will be decreased. The proportion of nitrogen in the non-volatile ether extract of pure pepper is remarkably constant, varying ordinarily between 3.75 and 4.10 parts per 100.

The starch by diastase, and copper-reducing substances by direct acid hydrolysis are both highest in the best grades of black pepper and lowest in the cheapest grades, while the reverse is true of crude fiber, total ash, and ash insoluble in acid. The difference between starch by acid and starch by diastase is not nearly so great in pure pepper, especially in white pepper, as in many of the common adulterants (see Table 66). The term *starch*, as used in the standards, has reference to the diastase method of determination, and inspection of Table 66 will show how easily the presence of some of the adulterants might be overlooked if the simpler method of direct hydrolysis by acid were relied upon entirely. The figures given in the tables indicate very clearly the importance of the crude fiber determination in showing adulteration, either with shells or hard vegetable substances.

The addition of small amounts of pepper shells is particularly hard to detect. The microscope is not of so much use, and there is obviously no chemical constituent of the shell which is not found in whole pepper. The examinations summarized in Table 65 were made especially to show the possibility of detecting this form of adulteration, and the table shows that the most valuable criteria for detecting shells are crude fiber, *d*-glucose (direct acid hydrolysis), magnesium oxide, the ratio of magnesium oxide to *d*-glucose, and the product of magnesium oxide and crude fiber. By comparison of these values and the authentic data given in the tables, if the variety of pepper under examination is known, it should be possible to detect the addition of as low as 10 per cent of shells. The use of pepper siftings in place of pepper shells is readily detected by the abnormally

high ash content without any marked change in the other significant values.

In the case of white pepper, determinations of ether extract and of nitrogen in the ether extract will again be found the best methods for detecting starchy adulterants. Pure white pepper should contain from 4 to 4.5 parts of nitrogen in 100 of ether extract. Such adulterants as olive stones, nutshells, and sawdust are indicated chemically by the low content of starch and high values for crude fiber.

For showing the actual character of the adulteration, the microscopical examination is, of course, of paramount importance.

TABLE 67.—ANALYSES OF ADULTERATED PEPPER

Variety	Total ash, per cent	Ash insol. in acid, per cent	Ether extract		Starch, per cent	Crude fiber, per cent	Microscopical
			Vol., per cent	Non-vol., per cent			
Black pepper...	3.45	0.35	0.3	9.1	32.9	19.4	Nutshells, cayenne, turmeric.
Black pepper...	6.2	0.85	0.12	6.42	46.4	14.4	Wheat, corn, buckwheat, mustard hulls.
Black pepper...	5.66	2.61	0.24	1.63	....	47.9	Olive stones and cayenne.
Black pepper...	9.65	2.26	0.11	8.20	31.16	16.2	Excess of shells.
White pepper..	2.25	......	.....	6.59	....	3.2	Wheat product.

In Table 67 are given a few typical analyses of adulterated pepper.

CASSIA AND CINNAMON

Although cassia and cinnamon are, as a matter of fact, obtained from different localities and from different species of plant, the terms have been employed so loosely that in common usage they have come to mean the same product. This interchangeability of the names is sanctioned to a certain degree by the standards of the Joint Committee on Definitions and Standards[1] in which Saigon cinnamon or cassia is defined as various species of the genus *Cinnamomum* apart from Ceylon cinnamon.

A study of the commercial product and its adulterations, however, would hardly be complete without pointing out what differences exist between the two spices.

[1] U. S. Dept. of Agr., *Food Inspection Decision* **172.**

The product ordinarily sold in the ground condition is undoubtedly cassia. Of this there are three principal varieties, Saigon, Canton or China, and Batavia cassia, consisting of the bark from various species of tree belonging to the laurel family.

Canton cassia is the poorest and cheapest grade, its average wholesale price being given as about half that of Batavia, the next higher, and only one-fifth that of Saigon, the most pungent and expensive of the cassias. The commercial bark, in the case of either variety, may come in large or small pieces, packed in bundles of several pounds weight, or in tightly rolled quills similar to those of true cinnamon but coarser. The latter form is more characteristic of Batavia cassia.

True cinnamon is the bark from the young branches of *Cinnamomum zeylanicum*, a small tree cultivated mainly in Ceylon. The bark is carefully removed from the branches, scraped, dried, and the thin pieces curled one within another into quill-like rolls. These are of a light-buff or brown color, streaked with lighter colored wavy lines of bast-fiber bundles, and are quite readily distinguished from the whole cassia.

Another portion of the cassia tree that is used as a spice is the dried immature fruit, known in commerce as *cassia buds*. They are occasionally used in powdered form as a spice directly but more commonly mixed with ground cassia bark. The buds themselves resemble cloves but are smaller and have the odor and flavor of cinnamon.

Composition.—Cassia and cinnamon contain a small quantity, 1 to 1.5 per cent, of essential oil, to which is mainly due their characteristic flavor. The oil is of a golden color when fresh, with an aromatic odor and a pungent taste, being powerful enough to blister the tongue. The important constituent of the oil is *cinnamic aldehyde*, $C_6H_5 - CH = CH - CHO$, of which 60 to 70 per cent is present.

Besides the oil the bark contains a considerable proportion of starch, some gum, resin, pigment, and a small amount of tannin, to the latter of which it doubtless owes its somewhat astringent taste.

Analyses of Authentic Samples.—The most extended series of analyses of genuine cassia and cinnamon are those made by

Winton, Ogden, and Mitchell[1] in their study of the composition of pure spices. These are summarized in the following table:

TABLE 68.—ANALYSES OF GENUINE CINNAMON AND CASSIA

Determination	Ceylon cinnamon			Cassia bark			Cassia buds
	Max., per cent.	Min., per cent.	Av., per cent.	Max., per cent.	Min., per cent.	Av., per cent.	Av., per cent.
Moisture	10.48	7.79	8.63	11.91	6.53	9.24	7.93
Total ash	5.99	4.16	4.82	6.20	3.01	4.73	4.64
Ash soluble in water.......	2.71	1.40	1.87	2.52	0.71	1.68	2.88
Ash insoluble in acid.......	0.58	0.02	0.13	2.42	0.02	0.56	0.27
Volatile ether extract......	1.62	0.72	1.39	5.15	0.93	2.61	3.88
Non-volatile ether extract..	1.68	1.35	1.44	4.13	1.32	2.12	5.96
"Starch" by acid..........	22.00	16.65	19.30	32.04	16.65	23.32	10.71
Crude fiber...............	38.48	34.38	36.20	28.80	17.03	22.96	13.35

The samples reported in the above table were above the average grade and hardly show the variations that may be met in the commercial powdered spice. Owing to varying conditions in the spice market, which may affect the quantity of a standard

TABLE 69.—ANALYSES OF COMMERCIAL CASSIAS

Variety	Total ash per cent.	Water-soluble ash per cent.	Ash insoluble in acid per cent.	Volatile ether extract per cent.	Non-volatile ether extract per cent.	Crude fiber per cent.
Seychelle bark (a).......	4.08	2.54	0.29	0.66	1.87	49.49
Seychelle bark (b).......	5.49	2.73	0.07	0.70	1.99	44.66
Ordinary broken China..	3.96	0.91	1.24	0.90	2.91	24.84
No. 1 broken Saigon.....	3.77	1.25	0.05	3.39	4.13	25.29
Extra No. 1 Batavia....	2.92	0.71	0.09	2.45	2.95	13.33
Pakhoi rolls............	2.62	0.82	0.33	1.16	2.58	21.07
Coarse Corintjie........	3.14	1.09	0.48	2.23	3.52	28.16
No. 1 Corintjie........	5.97	2.08	0.13	1.33	4.45	19.04
China rolls............	2.85	0.64	0.15	1.64	3.32	24.73
Good short Batavia.....	4.10	1.67	0.19	2.49	4.10	14.08
Kwangsi rolls 3d........	3.39	1.45	0.21	2.71	4.45	18.61

grade available and its price, the spice grinder is frequently obliged to use small quantities of broken or low-grade spice, blending them in such a way as to get a product that will conform to the legal requirements and at the same time possess the flavor demanded by his customers.

[1] *Conn. Agr. Expt. Sta., Ann. Rept.*, **1898**, 204.

Table 69[1] shows the variation in composition that may be met in cassias of this kind. The samples were all ground in small lots from the imported whole spice of the varieties named.

METHODS OF ANALYSIS

The usual determinations of total and soluble ash, ether extract, starch, and crude fiber are made as described on pages 387 to 389. It should be noted in the determination of starch that, since some samples of cassia form with water or dilute alcohol a glutinous mass that clogs the filter, it is best with this spice to
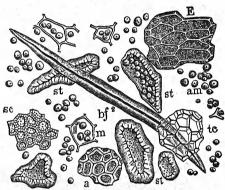
FIG. 67.—China Cassia. Elements of powder. *E*, epidermis; *st*, stone cells; *am*, starch grains; *sc*, stone cork; *tc*, thin cork; *bf*[2], bast fibers. × 160. (*A. L. Winton. Reprinted, by permission, from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

omit washing previous to the acid conversion. The determination of calcium oxalate has been recommended as of value in distinguishing between cassia and cinnamon (see page 406). It may be carried out as follows:

Calcium Oxalate.—Digest 5 grams of the powdered sample with an excess of dilute hydrochloric acid and filter. Evaporate the filtrate to small volume, filter again if necessary, nearly neutralize with ammonia, heat to boiling, and add ammonia in slight excess. Acidify with acetic acid and keep at the boiling point for some time. Filter, wash, and ignite strongly to CaO.

Microscopical Examination.—A summary of the microscopical characteristics of cassia and of its common adulterants is given in Chap. II, page 59. Figure 67 shows the elements of powdered

[1] SINDALL: *Ind. Eng. Chem.*, **1912**, 590.

cassia as drawn by Winton. Of these the most important are the spindle-shaped bast fibers, bf^2, with their narrow lumen or
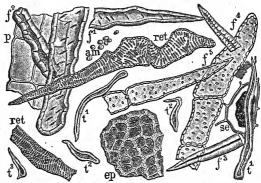
FIG. 68.—Cassia Buds. Elements of calyx. *ep*, epiderm; t^1, t^2, t^3, hairs; f^1, f^2, f^3, f^4, unicellular bast fibers; f^5, multicellular fiber; *ret*, reticulated vessels; *am*, starch grains. × 160. (*A. L. Winton. Reprinted, by permission, from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)
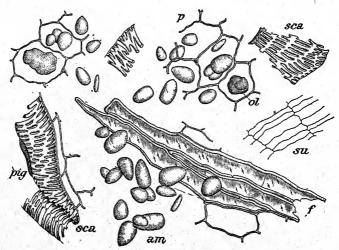
FIG. 69.—Ginger. Elements of powder. *su*, cork; *p*, parenchyma with starch grains and *ol*, oleoresin; *sca*, scalariform vessels; *f*, bast fibers; *am*, starch grains. × 160. (*K. B. Winton. Reprinted, by permission, from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

central canal (also Fig. 105, page 591); stone cells, *st*; starch grains, *am*, either singly or in aggregates of two or three. Possibly cork cells, *sc*, *tc*, depending on whether the bark has

been scraped or not, may be present. The harder tissues can be studied best after clearing with alkali or chloral hydrate.

Cassia buds do not differ greatly from cassia bark but can be distinguished best by the presence of the trichomes or short, crooked hairs shown at *a* in Fig. 108, page 592.

Figure 68 of cassia buds as drawn by Winton shows plainly various forms of these distorted hairs, t^1, t^2, t^3, as well as the reticulated vessels, *ret*, and multicellular fibers, f^5, consisting of a series of elongated stone cells, end to end, the terminal ones being pointed.

The vessels of ginger, Fig. 69, which has been found as an adulterant, somewhat resemble those of cassia buds, but the starch grains of ginger are entirely different from those of cassia and cassia buds.

INTERPRETATION OF RESULTS

The Federal standard for cinnamon and cassia is as follows: "*Ground cinnamon, ground cassia,* is the powder made from cinnamon. It contains not more than 5 per cent of total ash, nor more than 2 per cent of ash insoluble in hydrochloric acid."

The addition of such adulterants as ground bark or sawdust or of cereals would be indicated chemically by the high crude fiber in the first case and the decreased fiber and ether extract in the latter, if these adulterants were used singly. It will readily be seen, however, that the employment of mixtures of these substances, especially when other spices, as allspice or cayenne, are judiciously added, would be extremely difficult to detect by chemical methods. They would, on the other hand, be readily apparent in the microscopical examination.

Cassia buds have a higher percentage of non-volatile ether extract, and a lower percentage of crude fiber, than the bark, but otherwise are not essentially different in composition.

Distinction between Cassia and Cinnamon.—Cassia and cinnamon are so closely allied that to distinguish between them is difficult when they are in the form of powder. Cinnamon is lighter in color than cassia, the starch grains are only about half as large, and the bast fibers are somewhat narrower and more numerous. These differences, although noticeable by direct comparison with typical specimens, are, however, rather slight on which to base the actual identification of commercial

samples, especially when mixtures may be expected also. Winton[1] has summarized succinctly the essential microscopical differences in the cassia barks as distinguished from Ceylon cinnamon.

The crude fiber of cinnamon is higher than that of cassia, averaging 36.20 per cent, with a minimum of 34.38 per cent as against an average of 22.96 per cent and a maximum of 28.80 per cent for cassia (see Table 68); but this difference would be less noticeable with low-grade or only partially cleaned samples. A better distinction has been pointed out by Hendrick[2] in the content of calcium oxalate, which was found to vary from 0.05 to 1.34 per cent in cassia, and from 2.50 to 3.81 per cent in Ceylon cinnamon. Wild cinnamon contains even more calcium oxalate, two samples showing 6.62 and 6.99 per cent.

In view of the definition of cassia and cinnamon quoted on page 400, however, the distinction possesses more scientific interest than legal value, unless the samples were definitely labeled Ceylon or true cinnamon.

Cloves

Cloves are the unexpanded flower buds of *Eugenia caryophyllata* Thbg., or *Caryophyllus aromaticus* L., an evergreen tree of the myrtle family, the name of the spice coming from the French word "clou," meaning "a nail," which it somewhat resembles.

The clove tree is indigenous to the Moluccas or so-called "Spice Islands," but is now cultivated in Guiana, Ceylon, India, Zanzibar, and the East and West Indies. The chief varieties of commerce, named from their place of growth and graded in value in the order named, are Penang, Amboyna, and Zanzibar. The flowers are of a delicate pink color and grow in clusters of from nine to fifteen. During growth the green buds change to red and are then ready for harvesting. If they are allowed to remain on the tree several weeks longer they swell, forming an oblong berry containing several seeds. The fruit is then ripe and is known as the "mother clove" or clove fruit. It will have lost its pungency at this point, however, so that it is only the unripe buds that are gathered. At the proper time the buds are hand-picked or beaten from the tree with slender reeds, cloths being spread beneath to catch them. The only further treatment

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[1] "Structure and Composition of Foods," Vol. IV, p. 260.

[2] *Analyst*, **1907**, 14.

they receive is drying in the sun, which changes them to a rich brown color, or occasionally smoking over a fire, which imparts to the product a much darker tint.

The clove has a long cylindrical calyx, dividing above into four pointed spreading sepals, which surround four petals or leaves that are the unexpanded flowers. These are rolled into a globular bud at the head of the clove. The parts may be seen by soaking the clove in water, when the leaves will soften and unroll. The lower end of the calyx, of a deep, rich-brown color, is solid, with a dull wrinkled surface and dense fleshy texture. It abounds in essential oil which exudes on simple pressure with the finger nail.

General Composition.—Cloves are somewhat different from the species that have just been discussed with respect to the very large proportion of volatile oil that is present, a larger percentage of essential oil, in fact, than is found in any other natural food product. In addition there are found also resin, tannin, and a small amount of albuminoids. No starch is present. The average composition might be summarized as below:

	Per cent
Water	8.5
Ash	6.0
Essential oil	16.5
Fixed oil and resin	7.5
Tannin	18.0
Albuminoids	6.0
Fiber and cellulose	8.0

The most interesting constituent is the volatile oil, which may be readily obtained by distillation with steam and has a pungent taste and the characteristic odor of cloves. It consists mainly of *eugenol*, $C_{10}H_{12}O_2$, the amount present being from 80 to 95 per cent. Small quantities of eugenol esters and of the sesquiterpene, *caryophyllene*, are also found in the oil.

Authentic Analyses.—Analyses of genuine cloves, including the several varieties, have been reported by Richardson,[1] McGill,[2] and Winton, Ogden, and Mitchell.[3] Of these the analyses by the latter authorities, made by the same methods that were employed for the spices previously discussed, are given in Table 70.

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **13**, Part II, p. 225.
[2] Canada Inland Rev. Dept., *Bull.* **73**, p. 6.
[3] *Conn. Agr. Expt. Sta., Ann. Rept.,* **1898,** 206.

TABLE 70.—ANALYSES OF GENUINE CLOVES

| Determination | Cloves | | | Clove stems |
	Maximum per cent	Minimum per cent	Average per cent	Average per cent
Moisture	8.26	7.03	7.81	8.74
Total ash	6.22	5.28	5.92	7.99
Water-soluble ash	3.75	3.25	3.58	4.26
Ash insoluble in acid	0.13	0.00	0.06	0.60
Volatile ether extract	20.53	17.82	19.18	5.00
Non-volatile ether extract	6.67	6.24	6.49	3.83
"Starch" by acid	9.63	8.19	8.99	14.13
Starch by diastase	3.15	2.08	2.74	2.17
Crude fiber	9.02	7.06	8.10	18.71
Total nitrogen	1.13	0.94	0.99	0.94
"Oxygen equivalent"	2.63	2.08	2.33	2.40

McGill's analyses of genuine whole cloves, which are somewhat similar but include a greater number of samples, are summarized in the following table:

TABLE 71.—ANALYSES OF DIFFERENT VARIETIES OF CLOVES

Variety	Moisture per cent.	Volatile oil per cent.	Petroleum ether extract per cent.	Fixed oil per cent.
Penang:				
Maximum	7.4	24.3	28.2	12.0
Minimum	5.0	20.7	24.4	9.5
Average	6.2	22.4	27.0	10.8
Amboyna:				
Maximum	6.7	25.9	29.2	10.0
Minimum	5.5	23.5	26.5	8.2
Average	6.1	24.6	27.5	9.0
Zanzibar:				
Maximum	6.7	23.6	28.1	10.7
Minimum	4.1	18.6	21.3	8.0
Average	5.7	21.7	25.5	9.6

In making the analyses reported in this table the *moisture* was determined by drying the ground sample over sulphuric acid *in vacuo* for 24 hours; the total *volatile matter* by drying the ground sample at 98°C. for 18 hours; and the *petroleum ether extract* by the usual methods in a continuous extraction apparatus. The

volatile oil was obtained by subtracting the moisture from the total volatile matter.

Methods of Analysis

Moisture, ash, volatile and non-volatile ether extract, starch, and crude fiber are determined by the methods already described on pages 387 to 389.

With a product containing so much volatile oil the moisture is best determined by McGill's method,[1] in which a weighed portion (2 grams) of the ground sample is kept over sulphuric acid at about 60-mm. pressure for 24 hours, by which the whole of the aqueous vapor is absorbed by the sulphuric acid while only traces of the essential oil are removed.

Volatile Oil.—In the case of cloves and similar spices, as nutmeg and mace, high in volatile oil, it is recognized that the volatile ether extract is not a true measure of the oil, since there is considerable loss during the various stages of the lengthy determination. D i r e c t measurement of the oil is possible and offers the advantage that further tests can be made on the recovered oil to determine its purity.
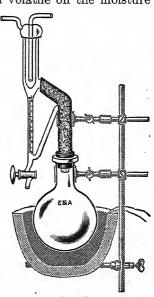
Fig. 70.—Distilling apparatus for volatile oils. (*Courtesy of Eimer & Amend.*)

Method.[2]—Transfer a weighed quantity of whole or ground material, sufficient to yield, if possible, 2 cc. or more of volatile oil, to a 500- to 2000-cc. round-bottomed, short-necked flask. Add to the flask 3 to 6 times as much water as material and mix uniformly. Set up the apparatus as indicated in Fig. 70, using the appropriate volatile-oil trap illustrated in Fig. 71. With an oil bath (hydrogenated cottonseed oil is satisfactory) as the source of heat, boil the contents of the flask slowly 4 to 8 hours, or until all the volatile oil has been distilled, taking care to avoid

[1] Canada Inland Rev. Dept., *Bull.* **73.**

[2] Clevenger: *J. Assoc. Off. Agr. Chem.*, **1934,** 70, 372.

the escape of vapors around the condenser. (With spices, for example nutmeg, containing volatile oils lighter than water and also fixed oils heavier than water, discontinue the distillation when the fraction of oil obtained during a 1 hour period is heavier than water.)

In the case of unsatisfactory separation of the volatile oil, draw off the contents of the trap into a small separatory funnel.

For Oils Lighter
Than Water

For Oils Heavier
Than Water
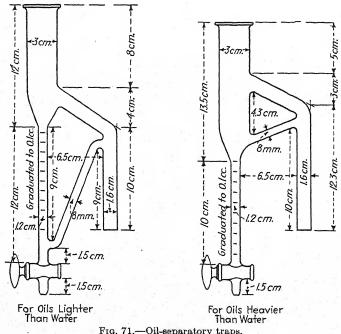
Fig. 71.—Oil-separatory traps.

After separation return the water to the trap and transfer the volatile oil to a graduated cylinder. Repeat the procedure if necessary.

With volatile oils heavier than water, after transferring to the graduated cylinder run the water with any remaining oil into a small separatory funnel. Wash the oil trap with 10 cc. of ether and transfer the washings to the funnel. Shake, and draw off the ether. Evaporate the ether and drain the residue into the cylinder. Read the quantity of volatile oil directly in the cylinder and report in terms of cubic centimeters per 100 grams of spice.

After allowing the oil to stand until perfectly clear or drying it with a minimum amount of anhydrous sodium sulphate and allowing it to settle, such tests as the specific gravity, optical rotation, refractive index, and eugenol content may be made, using for the most part semi-micro methods.

Tannin.—Since the quantity of tannin present in cloves is fairly constant and considerably higher than in the common adulterants or other spices, the determination is of some value for this spice. The usual method of oxidation by potassium permanganate may be used, having previously removed the easily oxidized oil by extraction with ether.

Reagents.—*a. Tenth-normal potassium permanganate solution,* which may be standardized against ferrous ammonium sulphate or pure oxalic acid as described in any standard book on quantitative analysis.

b. Indigo solution, made by dissolving 0.6 gram of the best sodium sulphindigotate[1] in 50 cc. of water, with the aid of heat, cooling, adding 5 cc. of concentrated sulphuric acid, making up to 100 cc., and filtering.

Process.—Extract 2 grams of the sample with anhydrous ethyl ether for 20 hours, or use the residue from the determination of "ether extract" if desired. In either case boil the residue for 2 hours with 300 cc. of water, cool, make up to 500 cc., and filter.

Measure 25 cc. of the filtrate into a 12-in. porcelain evaporating dish. Add 20 cc. (measured) of the indigo solution and 750 cc. of distilled water. Titrate the solution with the permanganate, stirring thoroughly, until the blue color of the solution begins to change to green, then add the permanganate a few drops at a time until the color changes to greenish yellow. Allow the liquid to stand for a moment or two, and then add the permanganate a drop at a time with thorough mixing until a bright golden-yellow color is obtained.

In the same way note the number of cubic centimeters of permanganate required to oxidize the 20 cc. of indigo solution alone. This amount, subtracted from the previous reading, leaves the quantity of permanganate needed to oxidize the tannin.

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[1] Only the purest indigo salt should be used since otherwise the end point is indefinite. The indigocarmin prepared by G. Gruebler and Co. of Leipsic and sold by dealers in microscopic staining material is well adapted for the purpose.

The result may be expressed as "oxygen equivalent," "oxygen absorbed" or directly as quercitannic acid. One cubic centimeter of $0.1N$ permanganate is equivalent to 0.0008 gram of oxygen, or from Neubauer's value for the reduction-equivalent of oak-bark tannin, to 0.006235 gram of quercitannic acid.

Notes.—The method is based on the fact, first worked out by Lowenthal,[1] that tannin is oxidized in acid solution by permanganate. The oxidation, however, proceeds slowly, and the end point is indefinite. By the addition of a considerable quantity of indigo the oxidation of the tannin is controlled, and the end point can be recognized by the change in color.

In order to ensure a uniform limiting action, the volume of permanganate used in a titration should not greatly exceed $1\frac{1}{2}$ times that which is required for the indigo alone, and the titrations should be carried out in a strictly similar manner as regards stirring and rate of addition of the permanganate.

It should be pointed out that, as described, the method does not determine the actual amount of tannin, since some other oxidizable substances still remain, although the greater part are removed by the extraction with ether. The more exact determination, however, involving the use of gelatin or hide powder to separate the tannin, would be much more tedious, and the results would be no more useful.

The value of the method is indicated by the following figures obtained with cloves and various adulterants.

Materials	Per cent. of "oxygen absorbed"	Per cent. of "quercitannic acid"
Cloves	2.35	18.5
Clove stems	2.40	18.8
Almond shells	0.40	3.2
Date stones	0.61	4.7
Spruce sawdust	0.30	2.4
Coconut shells	0.47	3.7
Linseed meal	1.00	7.8
Cocoa shells	1.26	9.7

Microscopical Examination.—Powdered cloves, under the microscope, differ somewhat from the typical spices that have been considered previously in that practically no characteristic

[1] *Z. anal. Chem.,* **1877,** 33.

elements can be seen without close study. The general appearance is that of a confused mass of dark-colored cellular tissue. Careful rubbing out under the cover glass between the thumb and finger, as described on page 48, will help in breaking up the masses, and treatment of the slide with chloral hydrate, as explained on page 49, will be of value. Even under the best conditions, however, the only special elements that will be readily apparent are the bast fibers, of which cloves contain a few. This lack of distinctly characteristic microscopical elements is in a way an advantage in that it renders the detection of foreign tissues easier.

The common adulterants that the microscope will show are clove stems, mother cloves, nutshells, fruit stones, allspice, and cereals.

FIG. 72.—Clove Stems. st^1, hypodermal stone cells; st^2, st^3, stone cells, and f^1, bast fiber outside of bundle ring; st^4 and f^2, stone cells and bast fiber inside of bundle ring; r, reticulated cells; sc, scalariform vessels; sp, spiral vessels; s, sieve tube. × 160. (*A. L. Winton. Reprinted by permission from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

Clove stems are distinguished from cloves by the greater number of bast fibers present, by the numerous stone cells (cloves have practically none) which much resemble those of allspice (Fig. 98, page 590) but are not accompanied by the lumps of colored resin; and especially by the characteristic bundles of reticulated and scalariform vessels shown in Figs. 99 and 110, pages 590 and 592.

Figure 72 is a drawing of the elements of clove stems, illustrating clearly the stone cells, st, bast fibers, f', f^2, and the reticulated, r, and scalariform, sc, vessels. The bast fibers and scalariform vessels are decidedly more woody and prominent than in cloves.

Mother cloves are characterized by the presence of large starch grains, am, Fig. 73, resembling quite closely those of sago, club-shaped or ovate, ranging up to 40μ in length, often having a distinct hilum in the broad end. Distinct crosses appear in polarized light.

Nutshells and *fruit stones*, typical examples of which are coconut shells and olive stones, are recognized by their char-

acteristic long and spindle-shaped stone cells as indicated under Allspice, page 59.

Allspice itself, being consider-ably cheaper than cloves and resembling the latter spice both in appearance and odor, is a favorite adulterant. Its charac-teristic elements are outlined on page 58, the feature best adapted for showing its presence
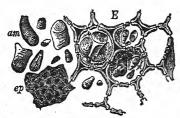
Fig. 73.—Mother Cloves. Ele-ments of seed. *ep*, epidermis, and *E*, ground tissue of cotyledon; *am*, starch grains. × 225. (*Moeller.*)

in cloves being the colored resin. Figure 111, page 592, is a photograph of cloves adulterated with ground allspice.

Other less characteristic adulterants, as ginger or the cereals, are also to be looked for and can be identified by the description recorded in Chap. II.

Interpretation of Results

The Federal standard for cloves[1] defines them as "the dried flower buds of *Caryophyllus aromaticus* L."

"They contain not more than 5 per cent of clove stems, not less than 15 per cent of volatile ether extract, not less than 12 per cent of quercitannic acid (calculated from the total oxygen absorbed by the aqueous extract), not more than 10 per cent of crude fiber, not more than 7 per cent of total ash, nor more than 0.5 per cent of ash insoluble in hydrochloric acid."

McGill,[2] as a result of a study of ground cloves sold in Canada and comprising 140 samples, suggests standards similar to the above, except that in place of the volatile ether extract, the minimum for volatile oil (see page 408) is fixed at 14 per cent.

Since the standards, however, are designed to include all samples of reasonable purity to which no additions of foreign material have been made, it will be found that the larger pro-portion of genuine samples will fall well within these limits. McGill, for example, points out that the ash of pure cloves will, in general, fall within 6 per cent and the acid-insoluble

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[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[2] Canada Inland Rev. Dept., *Bull.* **252**, p. 5.

ash within 0.3 per cent. This is equivalent to saying that the majority of genuine samples, and certainly those of high grade, will not approach the extreme limits of the standards.

The most valuable chemical determination is undoubtedly the volatile oil or volatile ether extract, and the result of this test, taken together with the ash data and possibly the tannin, will usually serve to indicate the extent to which any non-starchy adulterant has been added. Since cloves contain no starch, testing with iodine solution will show the presence of added starchy material, and the test may be readily confirmed by the microscopical examination.

Clove stems, a common adulterant, have only one-fourth as much volatile oil, half as much non-volatile ether extract, and more than twice as much crude fiber as cloves. The presence of stems as an adulterant also notably increases the ash and the acid-insoluble ash.

The presence of exhausted cloves is most readily shown by the decreased percentage of essential oil or volatile ether extract. The exhausted cloves, if whole, can be recognized by their shriveled and striated appearance, as well as the very dark, almost black color. According to Parry[1] the use of exhausted cloves is greater than might be imagined, one essential oil distillery, to the author's knowledge, having an annual output of 40 tons of such material.

Quite similar results to those obtained by the admixture of exhausted cloves, *i.e.*, a decrease in the volatile ether extract, soluble ash, and tannin, would be shown by a mixture of allspice with cloves, a form of adulteration that is not at all uncommon. This is shown clearly in the average figures for allspice given below. The characteristic tissues of allspice, however, render its detection by means of the microscope comparatively easy.

COMPOSITION OF ALLSPICE

	Per cent
Total ash..	4.50
Ash soluble in water.............................	2.50
Volatile ether extract............................	4.00
Non-volatile ether extract........................	5.75
Starch by diastase...............................	3.00
Crude fiber.....................................	22.00
Oxygen equivalent...............................	1.20
Quercitannic acid...............................	9.7

[1] "Food and Drugs," Vol. I, p. 222.

Artificial cloves made from dough, powdered bark, and clove powder, and from soft wood stained a dark color and soaked in a solution of oil of cloves, have been reported, but although interesting are too rare to be of any importance. . Apart from the detection of exhausted cloves, the microscope must be regarded as the chief reliance of the analyst in the examination of cloves.

MUSTARD

Source.—The mustard of commerce is the seed, whole or powdered, of several cruciferous plants of the genus *Brassica*. It is an annual herb, from 3 to 6 ft. high, with a yellowish flower and a small pod containing round seeds, and is a common plant in both Europe and America.

The plant is cultivated for spice largely in Bohemia, Holland, and Italy, certain parts of England, and in California and Kentucky of the United States.

Although numerous varieties of the mustard plant are known, only two are of commercial importance, black or brown mustard, *Brassica nigra*, of which the best comes from Italy through the port of Trieste, and white or yellow mustard, *Brassica alba*, the best grades of which come from England and Holland.

Manufacture.—Mustard seed contains so much oil that it cannot be ground between stones in the ordinary manner of grinding spices. It is crushed by passing between rollers, then pressed by hydraulic presses to extract the oil, after which the dried residue or "mustard cake" is put into pots and pounded by powerful stamps until the material is reduced to the desired powder. It is then sifted on sieves of silk cloth to separate the coarse hulls from the fine flour. The yield of flour varies from 30 to 60 per cent of the weight of the seed. The residue on the sieves is frequently used in the manufacture of "prepared" or "French" mustard, a paste composed ordinarily of ground mustard seed, salt, spices, and vinegar.

Composition.—The general composition of mustard seed is shown in the analysis of black and white mustards by Piesse and Stansell[1] in the table on page 417.

Both varieties of mustard are thus seen to contain considerable quantities of non-volatile oil, albuminoids, and mucilaginous material, but no starch. This fixed oil is entirely lacking in

[1] *Analyst*, **1880**, 163.

Determination	White mustard, per cent	Black mustard, per cent
Water..........................	8.00	8.52
Non-volatile oil..................	27.51	25.54
Cellulose.......................	8.87	9.01
Albuminoids.....................	28.06	26.50
Myrosin and albumin.............	4.58	6.46
Soluble matter...................	26.29	24.22
Volatile oil.....................	0.08	0.47
Ash...........................	4.70	4.98

pungency, being a bland, tasteless oil somewhat resembling olive oil, and is to some extent used as an adulterant of the latter (see page 201).

The interesting constituent of the mustard is the volatile oil. This is not present in the mustard as such but is developed by the addition of cold water. The actual substance present is *sinigrin* or potassium myronate ($KC_{10}H_{16}NS_2O_9$), a glucoside which by hydrolysis, through the agency of the enzymes also present, splits into glucose, potassium acid sulphate, and allyl isothiocyanate (C_3H_5CNS). The latter is the pungent volatile mustard oil. The reaction may be expressed:

$$KC_{10}H_{16}NS_2O_9 + H_2O \rightarrow C_6H_{12}O_6 + HKSO_4 + C_3H_5CNS.$$

White mustard contains a somewhat similar glucoside, *sinalbin* ($C_{30}H_{42}N_2S_2O_{15}$). This splits in an analogous manner to the sinigrin, forming *sinalbin mustard oil* (C_7H_7ONCS), which in pungency much resembles the volatile oil from black mustard.

Analyses of Authentic Samples.—The composition of mustard seed, flour, and hulls, as shown by the usual methods of analysis for spices, is summarized in Table 72.[1]

These mustard "flours" were prepared by the commercial methods, from seeds separated as thoroughly as possible from the hulls, and having the usual proportion of fixed oil removed by pressure.

ANALYTICAL METHODS

Tests for the amount of ash, ether extract, crude fiber, starch, etc., are made by the usual methods applicable to spices and already described.

[1] LEACH: *J. Am. Chem. Soc.*, **1904**, 1203.

These methods will ordinarily give all the needed information regarding the purity or quality of a sample of mustard. If, however, it is desired to make an actual determination of the amount of volatile mustard oil, the following method will be found as convenient as any.

TABLE 72.—ANALYSES OF GENUINE MUSTARD

Determination	Whole seeds			Flour			Hulls		
	Max. per cent	Min. per cent	Aver. per cent	Max. per cent	Min. per cent	Aver. per cent	Max. per cent	Min. per cent	Aver. per cent
Moisture.....................	6.82	5.88	6.37	9.50	5.09	6.96	9.12	5.36	7.48
Total ash.....................	4.83	3.84	4.25	5.58	4.66	5.03	5.03	4.43	4.65
Water-soluble ash..............	0.73	0.45	0.56	0.27	0.09	0.19	2.33	0.95	1.67
Ash insoluble in HCl............	0.56	0.16	0.32	0.50	0.08	0.27	0.22	0.04	0.12
Volatile ether extract...........	0.0	0.0	.....	0.0	0.0	.....	0.0	0.0	
Non-volatile ether extract.......	37.81	27.19	31.22	25.95	12.65	18.59	13.81	6.17	8.56
Total nitrogen.................	5.09	3.96	4.41	7.44	6.21	6.78	4.04	2.90	3.45
Crude fiber...................	6.53	4.21	5.04	3.28	1.87	2.42	18.95	10.90	15.20
"Starch" by acid hydrolysis.....	10.06	6.94	8.62	11.89	4.87	6.85	20.40	9.90	16.96
"Starch" by diastase...........	1.82	0.92	1.48	0.71	0.00	0.28	6.11	1.20	3.62
Alcohol extract................	17.75	13.70	15.50	25.31	19.22	22.30	14.21	8.07	11.07

Volatile Mustard Oil.—The method[1] depends on the fact that the mustard oil may be distilled into ammonia, forming thiosinamine,

$$C_3H_5CNS + NH_3 \rightarrow CS\diagdown_{NH_2}^{NH \cdot C_3H_5}$$

This latter when treated with silver nitrate precipitates an amount of silver sulphide corresponding to its content of sulphur, from which it may readily be determined. The method is:

Place 5 grams of the powdered mustard in a 200-cc. flask, add 100 cc. of water, stopper tightly, and macerate for 2 hours at about 37°C. Add 20 cc. of 95 per cent (by volume) alcohol and distill about 60 cc. into a 100-cc. graduated flask containing 10 cc. of 10 per cent ammonium hydroxide solution, taking care that the end of the condenser dips below the surface of the solution. Add 20 cc. of 0.1N silver nitrate solution to the distillate, set aside over night, heat to boiling on a water bath in order to flocculate the silver sulphide, cool, make up to the mark with water, and filter. Acidify 50 cc. of the filtrate with 5 cc. of concentrated nitric acid

[1] KUNTZE: *Ann. Chim. Anal.*, **1913**, 61.

and titrate with 0.1N ammonium thiocyanate, using 5 cc. of 10 per cent ferric alum as indicator. Each cubic centimeter of 0.1N silver nitrate used corresponds to 0.004956 gram of allyl isothiocyanate. If desired the result may be calculated to crude oil of mustard, containing 93 per cent of allyl isothiocyanate.

The Federal standards[1] require for the different varieties of black mustard seed a content of 0.6 per cent of volatile mustard oil, calculated as allyl isothiocyanate and determined by the above method.

Coloring Matter.—The presence of coal-tar dyes may be shown by digesting the sample for several hours with water containing a few drops of ammonia, filtering, acidifying very slightly with hydrochloric or acetic acid, and dyeing on wool, as described on page 70. If enough color is present it may be stripped from the wool and identified by the systematic procedure given in Table 4, page 75.

Turmeric may be detected by the microscope or in an alcoholic extract of the mustard by the boric acid test given on page 100.

Microscopical Examination.—The general characteristics of mustard flour, as viewed in the microscope, are summarized on page 62. Since it is common practice to mix the flour of the black and the white mustard, the commercial product will frequently exhibit the characteristics of both varieties. The bulk of the material consists of protein and fat, with occasional fragments of the palisade layer of the hull (Fig. 122, page 594), gray and black in the case of the white mustard and brown and black with black mustard. Groups of the mucilaginous epidermal cells (Fig. 121, page 594), and *ep*, Fig. 75, are also usually present.

Drawings of the elements of the seed of black (brown) and white mustard are shown in Figs. 74 and 75. The tissues most apparent in the ground material are these: Brown mustard, the inner portion of the palisade layer, pal^2, white mustard, the outer epidermis, *ep*, and the inner palisade layer, pal^2.

Turmeric is readily identified by the brilliant greenish-yellow color of the "paste balls," oleoresin cells that contain a deep-yellow dyestuff, *curcumin*, which becomes reddish brown if a little alkali is drawn under the cover glass.

[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

Since mustard contains no starch, the addition of cereals or similar adulterants can readily be shown by the iodine reaction and the different starches identified microscopically.
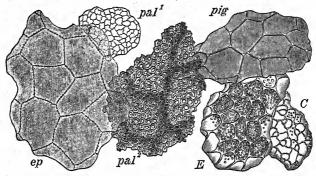
FIG. 74.—Black Mustard. Elements of seed. *ep*, outer epiderm; *pal*[1], outer portion of palisade layer; *pal*[2], inner portion of palisade layer; *pig*, pigment cells; *E*, endosperm. × 160. (*K. B. Winton. Reprinted by permission from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

A common adulterant is *charlock,* or wild mustard, which is found as a weed in the wheat fields of the Northwest. This is easily recognized by the fact that the palisade cells, which
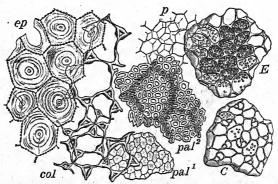
FIG. 75.—White Mustard. Elements of seed. *ep*, outer epiderm; *pal*[1], outer portion of palisade layer; *pal*[2], inner portion of palisade layer; *p*, parenchyma; *E*, endosperm. × 160. (*K. B. Winton. Reprinted by permission from "Structure and Composition of Foods" by Winton and Winton, John Wiley & Sons, Inc.*)

somewhat resemble those of genuine mustard, give a deep-red color when treated with chloral hydrate. To a small quantity of the material on a slide add 2 drops of a solution of 16 grams of

chloral hydrate and 1 cc. of strong hydrochloric acid in 10 cc. of water. Heat gently and examine with a low power. The palisade cells of charlock will become a deep blood red.

INTERPRETATION OF RESULTS

The Federal standards[1] define *"mustard flour," "ground mustard," "mustard,"* as a powder made from mustard seed with the hulls largely removed and with or without the removal of a portion of the fixed oil. It contains not more than 1.5 per cent of starch, nor more than 6 per cent of total ash.

It will be observed in this standard that the hulls must necessarily be removed in the preparation of the mustard flour. In the common acceptance of mustard flour, moreover, the presence of any considerable quantity of hulls would be considered an adulteration, and, indeed, reference to the table on page 418 indicates that by limiting the amount of starch that may be present the proportion of hulls is to a certain extent limited also. The other determinations that would be of the greatest value in showing the presence of excessive amounts of hulls are the soluble ash and the crude fiber.

Such adulterants as turmeric, cayenne, charlock, or cereals are mentioned under the heading Microscopical Examination, since this is by far the readiest means of showing their presence. The claim is sometimes made that turmeric is needed to tone down the pungency of the product and add to its keeping quality.[2] This, however, can be done by using a greater proportion of the yellow seed in the ground mixture.

Mineral adulterants are rarely seen nowadays, but if present would be readily shown in the ash determination.

The actual relative value of different samples as regards pungency can best be determined from the amount of allyl isothiocyanate produced when mixed with water.

A typical analysis of the "Dakota" wild mustard that is sometimes mixed with or substituted for the lower grade of genuine mustard is given on page 422. This sample was found by microscopical examination to be free from hulls, and the copper-reducing matter determined by diastase is actual starch.

[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[2] GIBBS, "The Spices and How to Know Them," p. 167.

Dakota Mustard Flour

	Per cent
Total ash	7.80
Soluble ash	0.46
Ash insoluble in acid	0.75
Ether extract	12.23
Total nitrogen	6.84
Volatile oil	3.76
Crude fiber	2.28
Starch by diastase	2.58

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CHAPTER IX

CIDER VINEGAR

General.—Cider vinegar may be defined as the product made by the alcoholic and subsequent acetic fermentation of fresh apple juice. The word "fresh" has a real significance in the definition, since the courts have held it illegal to sell under the name "cider vinegar" a product made from evaporated apples. Vinegar is made also by the similar fermentation of wine, malted cereals, or other saccharine substances, yielding *wine, malt,* or *sugar* vinegar according to the material employed. The acetic fermentation in the case of cider vinegar will take place spontaneously but is much aided by the addition of vinegar containing some of the "mother," a feltlike scum that forms on the surface of the cider during its change to vinegar and contains the various microorganisms that bring about the fermentation.

The process may be carried out by allowing the cider to stand for a long time in casks that are open to the air, but on a commercial scale the vinegar is usually made by allowing the alcoholic liquid from a large storage tank to trickle slowly through a column of shavings, rattan, or other fibrous material impregnated with old vinegar. A current of air is allowed to pass upward through the "generator" at the same time so that the fermentation is greatly hastened. The process is under exact control and yields a much more uniform product than is possible under the older barrel method.

The fermented product contains acetic, malic, and lactic acids, traces of alcohol, aldehydes, furfural and other volatile substances, as well as some of the unchanged sugar and the glycerol resulting from the fermentation.

Forms of Adulteration.—The adulteration of cider vinegar may consist in the substitution of a product almost entirely artificial, but imitating the genuine article in appearance and acidity; in the admixture or substitution of vinegar from another source, as glucose, or sugar vinegar, but so manipulated as to

conform to many of the analytical constants of genuine cider vinegar; finally, in the "improvement" of a low-grade or inferior product by the addition of cheap material so that it shall simulate the standard article. On account of the low price at which vinegar must be sold and the small margin of profit to the manufacturer, much ingenuity has been displayed in the manipulation of inferior grades so that they shall conform to the legal standards.

Typical practices consist in the addition of water in the reduction of vinegar of high acid content to legal standard, which is permissible only when declared on the label, and further, it may reduce not only the acid strength, but the amount of other ingredients in the same proportion. This means that other materials, high in solids and reducing sugars, must be added also, For this purpose, boiled cider, cheap cider jelly, or unfermented apple juice are commonly used. Dilute acetic acid, derived from the dry distillation of wood and not from the acetic fermentation of alcohol, may be used to bring back the acid strength. These rather crude methods may have to be helped out by the addition of mineral salts to furnish "ash" and "alkalinity." Even the careful addition of glycerol was practiced when this substance became of analytical importance.

Perhaps more common, however, is the admixture, or even occasional substitution, of distilled vinegar, called also "spirit" or "grain" vinegar, at one time frequently, but incorrectly, sold as "white wine vinegar," an entirely different article. This is made by submitting to acetic fermentation a dilute alcohol obtained by the fermentation with yeast and subsequent distillation of a mash of corn, malt, and rye. This distilled vinegar, colored with caramel, may be substituted entirely for the cider vinegar, but is usually mixed with the latter in varying amount and the analytical constants restored to normal by the addition of apple waste or boiled cider, high in solids and sugar. Diluted acetic acid, "artificial vinegar," which quite closely resembles distilled vinegar, may also be used.

Vinegar may be made from the cheaper grades of cane sugar or low-grade molasses which in many respects closely resembles cider vinegar and is much less easily detected than the admixture with distilled vinegar.

Certain waste products of the apple itself are also utilized in the manufacture of spurious cider vinegars, such as "apple

waste" from the skins, cores, and chops of the dried-apple industry. If carefully made, the resulting vinegar, which legally should be labeled "dried-apple vinegar," is not especially different in composition from genuine cider vinegar, although inferior from the standpoint of flavor through the loss of some of the natural ethereal flavoring matters of the apple by drying. The presence of sulphates, from the sulphur used during the drying process, formerly constituted a fairly good test for this product. Since, however, the sulphates may have been purposely removed by precipitation with barium salts, the spectroscopic test for the latter has more or less come into use as a test.

Traces of arsenic may be present from the arsenical spray used on the apples, but numerous tests by the Connecticut Agricultural Experiment Station and others have shown that the amount is ordinarily too small to be of significance.

"*Second Pressings.*"—In the manufacture of cider vinegar the "pomace" from which the apple juice has been expressed is sometimes allowed to stand in piles for several days, during which time it becomes much heated and a fermentation ensues, accompanied by partial decomposition, so that when the material is subjected again to high pressure a greater yield of material of rather questionable character is secured.

It is quite easy to imitate some of the characteristics of cider vinegar but difficult to alter all the analytical constants so that adulteration shall not be shown.

METHODS OF ANALYSIS

The sample should be thoroughly mixed before analysis and if turbid should be filtered. The results may be expressed as per cent by weight or as grams per 100 cc. The latter method is simpler and for general purposes entirely satisfactory.

Specific Gravity.—Determine at $\frac{20°}{20°}$C. as directed under General Methods, page 4.

Solids.—Measure 10 cc. into a weighed flat-bottomed platinum dish of 50 mm. bottom diameter, evaporate on a boiling water bath for 30 minutes, dry for 2½ hours at the temperature of boiling water, cool in a desiccator, and weigh.

Note.—This is the official method of the Association of Official Agricultural Chemists. It will give concordant results if dishes

of the size and shape specified are used and if the residue is dried for exactly the time stated. Owing, however, to the persistent retention of acetic acid in the solids, more accurate results are obtained by adding 5 cc. of water to the residue and again evaporating to dryness, making three evaporations in all before cooling and weighing.

Ash.—Measure 25 cc. of the vinegar into a weighed platinum dish, evaporate to dryness on the steam or water bath, and ignite in a muffle furnace for 30 minutes at between 500 and 550°C. Break up the charred mass in the dish, add hot water, filter through an ashless filter, and wash *thoroughly* with hot water. Return the filter and contents to the dish, dry, and ignite in the muffle at between 500 and 550°C. for 30 minutes, or until all the carbon is burned off. Add the filtrate, evaporate to dryness, and ignite at between 500 and 550°C. for 15 minutes. Cool in a desiccator and weigh. (Weight *A*.) Reheat in the muffle at between 500 and 550° for 5 minutes and cool for not more than 1 hour in a desiccator containing an efficient desiccant. Put only one dish in a desiccator at a time. Place Weight *A* on the balance pan before removing the dish from the desiccator and weigh rapidly to a milligram. Calculate the ash from the last weight.

Notes.—The ashing of vinegar is not a simple process if an accurate result is to be obtained. The range of temperature should be kept fairly close to that stated, although it may go up to 600° for a short time without serious loss. The effect on the phosphoric acid content, if the ash is to be used later for this determination, is more marked, increased temperature increasing the soluble and decreasing the insoluble phosphoric acid.

The ash from cider vinegar is markedly hygroscopic, owing to its content of potassium carbonate, and must be treated and weighed accordingly, especially if the humidity is high. If sulphuric acid is used in the desiccator it should be renewed frequently.

Solubility and Alkalinity of Ash.—Determine as directed on page 25. Express the result of the titration as the number of cubic centimeters of 0.1*N* acid per 100 cc. of sample.

Alcohol.—To 100 cc. of the vinegar add saturated sodium hydroxide until faintly alkaline and distill almost 50 cc. Excessive foaming may be prevented by a piece of paraffin the size

of a pea. Make up to 50 cc. at the temperature of the sample, filter if necessary, and determine the specific gravity with a pyknometer as on page 4. Calculate the per cent of alcohol by volume from the table on page 489.

Note.—The amount of alcohol is very slight, usually not over a few tenths of 1 per cent in a properly made vinegar, being really a measure of the thoroughness of the acetic fermentation.

Total Acidity.—Dilute 10 cc. with boiled and cooled distilled water until the solution is very light-colored, add phenolphthalein and titrate with $0.1N$ sodium hydroxide. Calculate as acetic acid, $HC_2H_3O_2$.

Non-volatile Acids.—Evaporate 10 cc. just to dryness in a porcelain dish on the water bath, add 5 to 10 cc. of water and again evaporate; repeat until at least five evaporations have been made and no odor of acetic acid can be detected. Add about 200 cc. of recently boiled and cooled distilled water, and titrate with $0.1N$ sodium hydroxide and phenolphthalein. Express the result as malic acid, $H_2C_4H_4O_5$.

Note.—Although it is customary to report the fixed acid as malic, it is undoubtedly true that the acid present is mostly lactic, the major portion of the malic acid being converted during the fermentation and acetification to lactic and carbonic acids.[1]

Volatile Acid.—Calculate the non-volatile acids as acetic and deduct from the total acid. Express the result as acetic acid.

Polarization.—If possible, polarize in a 200-mm. tube without decolorizing. Report results on basis of 200-mm. tube in Ventzke degrees. If necessary, decolorize as follows:

a. To 50 cc. of the sample add a measured amount of saturated neutral lead acetate solution, avoiding an excess. Filter, remove the lead with powdered potassium oxalate, and filter again. Polarize as before, correcting the reading for the dilution with the lead acetate solution.

b. To 50 cc. of the sample add 1 gram of decolorizing carbon, such as "Nuchar," "Norit," or "Darco." Filter through a double filter and polarize.

Notes.—It is best to avoid the use of basic lead acetate, the usual clarifier, because in the presence of malic or lactic acid the effect of the lead salts upon the rotation of these may lead

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[1] HARTMAN and TOLMAN: *Ind. Eng. Chem.*, **1917**, 759.

to erroneous results.[1]   Neutral lead acetate is not especially objectionable.   If lead is used it should be removed by potassium oxalate before polarization.   Excess of vegetable carbons should be avoided since they will cause a slight change in polarization toward the right.

The average apple juice contains about twice as much reducing sugar as it does sucrose, and the proportion of levulose is more than twice the dextrose.   Hence apple juice is strongly levo-rotatory, averaging about $-18°$ Ventzke.   During the alcoholic fermentation the sucrose quickly disappears, and also most of the dextrose, but the levulose persists in slight but distinct proportion to the end of the acetic fermentation, so that the natural polarization of a true cider vinegar is always levorotatory.

Total Reducing Substances before Inversion.—Measure 25 cc. of the sample into a 50-cc. volumetric flask and nearly neutralize with strong sodium hydroxide solution $(1 + 1)$.   Cool, make up to the mark, and determine the reducing substances in 20 cc. or more of the solution, depending upon the amount of reducing substances present, by the Munson and Walker method, page 266.   Report the result as invert sugar.

Note.—Vinegar contains varying amounts of volatile reducing substances, largely acetylmethylcarbinol, which should be eliminated by evaporation to get the true amount of sugar.   This may be done as follows: Evaporate 25 cc. to 5 cc. on the water bath.   Add 25 cc. of water and evaporate again to 5 cc.   Wash into a 100-cc. graduated flask, make up to the mark, and determine reducing sugar in 50 cc. by Fehling's solution as before.   Express the results as grams of invert sugar in 100 cc. of sample.

Total Reducing Substances after Inversion.—Invert 25 cc. of the vinegar in a 50-cc. volumetric flask with 5 cc. of hydrochloric acid, as described on page 293.   Neutralize with strong sodium hydroxide solution $(1 + 1)$, make up to the mark, and determine reducing substances by the Munson and Walker method as under Total Reducing Substances before Inversion.

Sugars.—Evaporate 50 cc. of the vinegar on the steam or water bath to a sirup, add 10 cc. of water, and evaporate again.   Repeat once more with 10 cc. of water.   Transfer the residue with about 50 cc. of warm water to a 100-cc. volumetric flask.   If the determination of total reducing substances before and after inversion

[1] Balcom and Yanovsky: *J. Assoc. Off. Agr. Chem.*, **1921**, 245.

has shown the absence of sucrose, make up to the mark and determine the reduction with Fehling's solution as before. If sucrose is found to be present, invert with hydrochloric acid and neutralize before diluting to volume and determining the copper reduction. In either case calculate the result as invert sugar.

Volatile Reducing Substances.—If sucrose is absent, subtract the non-volatile reducing substances from the average of the total reducing substances before and after inversion. If sucrose is present, subtract the non-volatile reducing substances from the total reducing substances after inversion.

Acetylmethylcarbinol.—As mentioned on page 428 the volatile reducing substances of vinegar consist largely of acetylmethyl-carbinol,[1] $CH_3CHOHCOCH_3$, a substance produced in many fermentations and found not only in silage, buttermilk, bread, and many other food products in which fermentation has occurred, but produced as well by the destructive alteration of carbo-hydrates. It is a normal constituent of cider vinegar.

Acetylmethylcarbinol can be determined approximately by its reducing action on Fehling's solution in the cold.[2] If the distilled sample is allowed to stand overnight with Fehling's solution, the weight of the precipitated cuprous oxide, multiplied by the factor 0.25, will give the approximate amount of acetyl-methylcarbinol. A more exact method is to oxidize it to diacetyl, which after distillation is converted by means of hydroxylamine to diacetyl-dioxime, the latter being precipitated with nickel sulphate as nickel dimethylglyoxime.

For most purposes, however, a simple direct determination of the volatile reducing substances will serve as well and is somewhat more positive than the result obtained above by difference. As suggested by Crawford,[3] dilute 50 cc. of the vinegar to 250 cc. and distill 200 cc. Neutralize the distillate, make up to 250 cc., and determine the copper reduction on 50 cc. by the Munson and Walker method (page 266). Express the result as grams of invert sugar per 100 cc. of vinegar on a basis of 4 per cent acidity.

Crawford found the amount in cider vinegar to be fairly constant, varying from 0.11 to 0.24 gram per 100 cc. Distilled

[1] BROWNE: *J. Am. Chem. Soc.,* **1903,** 29; PASTUREAU: *J. pharm. chim.,* **1905,** 593; BALCOM: *J. Am. Chem. Soc.,* **1917,** 309.

[2] ARBENZ: *Mitt. Lebensm. Hyg.* **1924,** 52.

[3] *Ind. Eng. Chem.,* **1913,** 845.

or artificial vinegar contains from a trace to 0.0026 gram per 100 cc. under the same conditions. Balcom by a similar method found 0.24 gram per 100 cc. for cider vinegar of known purity.

A somewhat similar determination is that of the oxidation value, page 431.

Soluble and Insoluble Phosphoric Acid.—For the *soluble phosphoric acid* use the solution left after determining the alkalinity of the soluble ash. Concentrate, if necessary, make up to 50 cc. in a graduated flask, and use 25 cc. Add 2 cc. of nitric acid (sp. gr. 1.20) and 10 grams of ammonium nitrate. Add 25 cc. of ammonium molybdate solution,[1] stir thoroughly, and place on a water bath at a temperature of 40 to 60°C. When clear, test 5 cc. with warm ammonium molybdate. If no further precipitation occurs, allow the solution to stand for 1 hour at 40 to 60°C., filter on a Gooch crucible, using an asbestos felt about $\frac{1}{4}$ in. thick, and wash twice with water, then with 2 per cent potassium nitrate solution until 10 cc. of the filtrate gives a pink color with 1 drop of $0.1N$ sodium hydroxide and phenolphthalein. Transfer the precipitate and the asbestos to the beaker used for the molybdate precipitation, add $0.1N$ sodium hydroxide until the precipitate is all dissolved and the solution colorless (25 cc. is ordinarily sufficient); add 1 cc. of phenolphthalein solution and titrate with $0.1N$ hydrochloric acid for the excess of alkali. Calculate the result as milligrams of P_2O_5 in 100 cc. of vinegar (1 cc. of $0.1N$ alkali = 0.0003088 gram P_2O_5). A blank should be run at the same time, using the same amount of reagent, in order to correct for any precipitation of the molybdate solution.

For the *insoluble phosphoric acid* exhaust the "insoluble ash" by boiling with several small portions of hot water acidulated with nitric acid, neutralize with ammonia, make up to 50 cc., take 25 cc., and proceed as in the determination of soluble phosphoric acid.

Note.—Too much stress should not be laid with different samples upon the relative amounts of soluble compared to

[1] Prepared by dissolving 100 grams of molybdic acid in 144 cc. of ammonium hydroxide (sp. gr. 0.90) and 271 cc. of water; slowly and with constant stirring pour the solution thus obtained into 489 cc. of nitric acid (sp. gr. 1.42) and 1,148 cc. of water. Keep the mixture in a warm place for several days or until a portion heated to 65°C. deposits no yellow precipitate of ammonium phosphomolybdate. Decant the solution from the sediment and preserve for use.

insoluble phosphoric acid, because this relation depends some-
what upon the temperature reached in ashing the vinegar, a
higher temperature resulting in a uniformly larger proportion of
soluble phosphoric acid.   This is probably due to incipient fusion
of the ash material, converting some insoluble phosphoric acid
to the soluble form.   The total amount of phosphoric acid,
however, is not appreciably affected.

Oxidation Value.—This and similar tests have been proposed
recently to distinguish more clearly between distilled vinegar
and diluted acetic acid or "artificial vinegar." The method
was at first applied to the vinegar itself after removal of the
coloring matter.[1] The results obtained in this way were not very
satisfactory and Edwards and Nanji[2] made an important
improvement in distilling the vinegar before making the test.
The process described here is that used by Edwards and Nanji
for malt vinegar and by Fisher for all samples. A measured
amount of the vinegar distillate is allowed to stand for a definite
time with an excess of standard permanganate and the excess
then determined by the liberation of iodine from potassium iodide
and titration with sodium thiosulphate.

Method.—Dilute 20 cc. of vinegar with 125 cc. of water and
distill 100 cc.   To 25 cc. of the distillate add 10 cc. of sulphuric
acid $(1 + 3)$ and 10 cc. (measured) of $0.1N$ potassium per-
manganate.   Allow the mixture to stand for exactly 30 minutes,
then add 5 cc. of 10 per cent potassium iodide solution, and titrate
the liberated iodine with $0.02N$ sodium thiosulphate, using starch
indicator.   Run a blank determination in exactly the same way,
using 25 cc. of water in place of the vinegar distillate.

If the volume of $0.02N$ sodium thiosulphate used in the blank
titration is A cc.. and that used in the titration of the distillate
is B cc., then

$$\text{Oxidation value} = 40(A - B)$$

With a fermentation vinegar, as malt or cider vinegar, dilute
5 cc. of the distillate with 20 cc. of water and proceed as before.
In this case the oxidation value $= 200(A - B)$.

[1] Pratolongo: *Ann. chim. applicata*, **1925**, 72; Schmidt: *Z. Unters.
Lebensm.*, **1935**, 472.

[2] *Analyst*, **1938**, 410; Fisher: *Conn. Agr. Expt. Sta., Ann. Rept.*, **1938**, 21;
Illing and Whittle: *Analyst*, **1939**, 329.

Notes.—The oxidation value is defined by Edwards and Nanji as: The cubic centimeters of $0.01N$ potassium permanganate used by 100 cc. of vinegar in 30 minutes under the standard conditions prescribed. Although the differences between the results found for distilled and artificial vinegars are considerable, since variations in the exact method followed will cause varying results, the analyst in any case of doubt should check his results with the published ones by tests on known samples. Since the vinegars reported by different analysts vary in acid strength, greater uniformity would be obtained also if the results were reported calculated to a definite acetic acid percentage.

The test is in general due to the distillation of small amounts of volatile oxidizable constituents, as alcohol, acetaldehyde, acetal, and acetylmethylcarbinol. Similar confirmatory tests, as oxidation with iodine instead of permanganate, and determination of saponifiable esters (ester value), give evidence of the same character, sometimes even more conclusive.

The average values found by Edwards and Nanji for artificial vinegars were 4.0 (0.8 to 6.4), for spirit vinegar (distilled vinegar) 153 (88 to 225), for distilled malt vinegar 946 (840 to 992). Fisher found 18 for diluted acetic acid and 830 for distilled vinegar known to be pure. Commercial distilled vinegars varied from 111 to 1,149. The few tests that have been reported for cider vinegar show very high values, about 3,500. The methods used were not quite the same in the two cases.

Storage of distilled vinegar of standard acidity (4 per cent) decreases the oxidation value, but there is only slight change on storing the strong (12 per cent acid) vinegar, indicating that the changes taking place are due to biological rather than chemical agencies.

Pentosans.—To 100 cc. of vinegar add 43 cc. of hydrochloric acid (sp. gr. 1.19) and carry out the distillation and determination as described on page 303.

Formic Acid.[1] *Method.*—Add 0.4 to 0.5 gram of tartaric acid to 100 cc. of vinegar and distill with steam. Pass the steam after leaving the vinegar through a boiling mixture of 15 grams of calcium carbonate and 100 cc. of water and keep this volume constant throughout the process. Collect 1,000 cc. of distillate

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[1] FINCKE: *Z. Nahr.-Genussm.*, **1911**, 1; **1912**, 88; SEEKER: *J. Assoc. Off. Agr. Chem.*, **1915**, 210, 556.

and reduce the volume of the sample to 30 to 40 cc. during the distillation. Reject the distillate. Filter and wash the calcium carbonate mixture, make the filtrate and washings, which should not exceed 140 cc. in volume, faintly acid with hydrochloric acid, add 10 to 15 cc. of mercuric chloride solution (10 grams of mercuric chloride and 3 grams of sodium chloride in 100 cc.) and heat in a boiling water bath for 2 hours. Filter on a Gooch crucible, wash with water, alcohol, and finally ether. Dry and weigh as HgCl. Factor for formic acid is 0.0977.
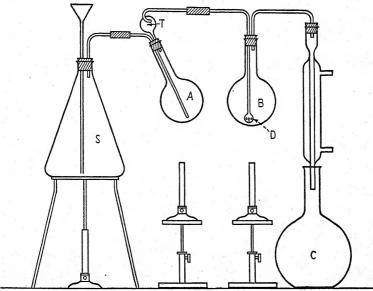
FIG. 76.—Apparatus for determination of formic acid.

Notes.—The following suggestions[1] will assist in obtaining successful results with the method: The apparatus may be set up as in an ordinary steam distillation, as in Fig. 76. A liter flask S, two-thirds filled with water and containing a few pieces of pumice that have been heated red hot and dropped into the water to ensure steady boiling, serves as a steam generator. The steam passes from this into a 330-cc. round-bottomed flask A with a short, wide neck, containing the sample to be tested. The delivery tube from this flask is of rather large bore, is pro-

[1] BENDER: U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, p. 78.

vided with a spray trap T, and leads to a pear-shaped liter flask B, with a neck not over 3 in. long. This flask contains the carbonate mix. The bottom of the entry tube leading into it should be blown into a bulb and pierced with 6 or 7 small holes (by means of the sharp red-hot end of a small file) arranged in a ring a short distance from the very bottom D. The entering steam is thus divided into many small bubbles and mixes thoroughly with the suspended carbonate. From this flask a delivery tube leads to a condenser and a liter flask C, simply for convenience and to judge the volume of the distillate. Restrict the volume of filtrate and washings from the carbonate mix to about 140 cc. Evaporate, if necessary, to this volume before acidulating. In heating the reaction mixture, immerse in the bath to the level of the liquid, but not over this level, and filter soon after the heating is completed. The distillation requires about 2 hours.

In this method, the greater part of the acetic acid and all the formic acid are retained in the carbonate flask as soluble calcium salts, and formic acid is determined by its reduction of mercuric chloride to the insoluble mercurous chloride. The method recovers 90 to 95 per cent of the formic acid, the principal error being in the distillation, since the precipitation of the calomel is quantitative.

Glycerol. *Solutions Required.* 1. *Strong Bichromate.*—Dissolve 74.56 grams of dry, recrystallized potassium bichromate in water, add 150 cc. of concentrated sulphuric acid, cool and make up to 1,000 cc. at 20°C.; 1 cc. of this solution equals 0.01 gram glycerol. The high coefficient of expansion of this strong solution necessitates careful volumetric measurement on account of the changes in room temperature from day to day. The solution has an apparent expansion in glass of 0.0005 (or 0.5 per cent) for each degree Centigrade. By observing this correction the solution may be measured if the room temperature varies from 20°C,[1] but it is better, if possible, to make all volumetric measurements at nearly 20°.

2. *Dilute Bichromate.*—Measure 25 cc. of the strong bichromate into a 500-cc. glass-stoppered volumetric flask, dilute with water,

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[1] More accurate results may be obtained, if necessary, by taking the specific gravity of the solution and using weighed quantities from a weight burette.

and make up to the mark at room temperature; 20 cc. of this solution is equivalent to 1 cc. of the strong bichromate.

3. *Ferrous Ammonium Sulphate.*—Dissolve 30 grams of crystallized ferrous ammonium sulphate in water, add 50 cc. of concentrated sulphuric acid, cool, and dilute to 1,000 cc. at room temperature; 1 cc. of this solution is approximately equivalent to 1 cc. of the dilute bichromate. Its value changes slightly from day to day, and it should be standardized against the dilute bichromate whenever used.

4. *Acid Retarding Solution.*—Dilute 150 cc. of sirupy phosphoric acid with 60 cc. of water and add cautiously 250 cc. of strong sulphuric acid.

Extraction.—Make all evaporations on a water bath, the temperature of which is maintained between 85 and 90°C. The area of the dish exposed to the bath should not be greater in circumference than that covered by the liquid inside. Evaporate 100 cc. of vinegar to about 5 cc., add 20 cc. of water, and again evaporate to about 5 cc. in order to expel acetic acid. Treat the residue with about 5 grams of fine sand (40 mesh) and with 15 cc. of milk of lime (containing about 15 per cent of calcium oxide), and evaporate almost to dryness with frequent stirring (avoid formation of dry crust or evaporation to complete dryness). Treat the moist residue with 5 cc. of hot water, rub into a homogeneous paste, and then add slowly 45 cc. of absolute alcohol, washing down the sides of the dish to remove adhering paste, and stir thoroughly. Heat the mixture on a water bath with constant stirring to incipient boiling, and decant the liquid through a 12.5-cm. fluted filter into a porcelain dish. Wash the residue twice by decantation, then repeatedly with small portions of hot 90 per cent alcohol, transfer all of the material to the filter, and continue washing until the filtrate amounts to about 150 cc., or, instead of filtering, centrifuge and wash three times. Evaporate to a sirupy consistency, add 10 cc. of absolute alcohol to dissolve the residue, and transfer to a 50-cc. glass-stoppered cylinder, using two additional portions of 5 cc. each of absolute alcohol to wash out the dish and complete the transfer. Add three portions of 10 cc. each of absolute ether, shaking thoroughly after each addition. Let stand until clear, then pour off through a filter, and wash the cylinder and filter with a mixture of 1 part of absolute alcohol to 1½ parts of absolute ether, pouring the

wash liquor also through the filter. If a heavy precipitate is observed in the cylinder, it is advisable to centrifuge at low speed and decant the clear liquid through a filter. Add 20 cc. of the mixture of absolute alcohol and absolute ether (1:1.5) to the precipitate in the cylinder, shake thoroughly, centrifuge, and decant; repeat this process three times. Evaporate the filtrate and washings to about 5 cc. on the water bath, add 20 cc. of water, and evaporate to about 5 cc.; again add 20 cc. of water and evaporate to about 5 cc.; finally add 10 cc. of water and evaporate to 5 cc. (These evaporations are necessary to remove all the ether and alcohol, and when conducted at 85 to 90°C. there is no loss of glycerol up to 50 per cent concentration.)

Transfer the residue with hot water to a 50-cc. volumetric flask, cool, add silver carbonate freshly precipitated from 0.1 gram of silver sulphate,[1] shake occasionally, and allow to stand 10 minutes; then add 0.5 cc. of basic lead acetate solution, page 289, shake occasionally, and allow to stand 10 minutes; make up to the mark, shake well, filter, rejecting the first portion of filtrate, and pipette off 25 cc. of the clear filtrate into a 250-cc. glass-stoppered volumetric flask. Add 1 cc. of concentrated sulphuric acid to precipitate the excess of lead (which otherwise would subsequently combine with part of the standard bichromate and cause an error). Then determine the glycerol by the following method:

Determination.[2]—Introduce into the 250-cc. flask, containing the 25-cc. purified glycerol solution, 30 cc. (measured) of the strong bichromate solution, carefully add 24 cc. of concentrated sulphuric acid, rotating the flask gently to mix its contents and avoid violent ebullition, then place in a boiling water bath for exactly 20 minutes. Remove the flask from the bath, dilute at once, cool, and make up to the mark at room temperature.

Standardize the ferrous ammonium sulphate solution by pipetting 20 cc. into a 250-cc. beaker, adding 20 cc. of the acid

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[1] Dissolve 0.1 gram of silver sulphate in about 50 cc. of water, add an excess of sodium carbonate solution, allow the precipitate to settle and wash with water several times by decantation until the washings are practically neutral. The reagent must be freshly prepared immediately before use.

[2] Hehner method, modified by Richardson and Jaffé, *J. Soc. Chem. Ind.*, **1898**, 330, and by Ross, U. S. Dept. Agr., *Bur. Chem. Bull.* **137**, p. 61.

retarding solution, 4 drops of diphenylamine indicator (1 gram of diphenylamine in 100 cc. of sulphuric acid), and about 100 cc. of water. Titrate with the dilute potassium bichromate solution until the liquid becomes dark green, then add the bichromate slowly drop by drop, stirring continuously, until the color changes from a blue gray to a deep violet. The number of cc. of dilute bichromate used = a.

In place of the dilute bichromate solution now substitute a burette containing the oxidized glycerol and excess of strong bichromate solution and titrate 20 cc. of the ferrous ammonium sulphate solution as before. Number of cubic centimeters used = b.

From the figures obtained calculate the glycerol, (grams per 100 cc. of vinegar), by the following formula:

$$G = 2 \times \left(B - \frac{250(a)}{20(b)} \right) \times 0.01,$$

where G = grams of glycerol per 100 cc. of vinegar, and B = cc of the strong potassium bichromate solution used to oxidize the glycerol.

Notes.—The volume of strong bichromate solution used must be sufficient to leave an excess of about 12.5 cc. at the end of the oxidation. Thirty cubic centimeters is enough for ordinary vinegars containing up to 0.35 gram of glycerol per 100 cc.

A slightly more accurate oxidation may be obtained by adding only 15 cc. of concentrated sulphuric acid and continuing the digestion for at least 2 hours in a boiling water bath.

The method is useful in showing the addition or substitution of distilled or artificial vinegar, neither of which contains glycerol, this having been found to be present in fairly constant proportion in the fermented product. Dilution with water would also naturally reduce the glycerol content. The determination is, however, time-consuming and affords little more information than the much easier determination of non-sugar solids or oxidation value. Moreover, it has been found entirely feasible by some producers to add glycerol directly. The determination is retained here partly because of its usefulness also in the examination of wines.

Interpretation of Results

The Federal standard for cider vinegar is as follows:[1]

"Vinegar, cider vinegar, apple vinegar, is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples, and contains, in 100 cubic centimeters (20°C.), not less than 4 grams of acetic acid."

Malt vinegar, wine vinegar, spirit vinegar, etc., are defined in various clauses of the above standard for vinegar, of which only the cider vinegar clause is given here. It is worthy of note that the one quoted is the only definition in which the word "vinegar," unqualified, is used. In the United States the only vinegar known for years was cider vinegar, and among food inspection officials it has always been held that when the customer simply asks for "vinegar," cider vinegar is called for and should be furnished. The word alone, then, indicates cider vinegar made from the fresh juice of apples.

An interesting phase of the use of so-called "standards" to facilitate the work of the official food analyst is shown in the case of vinegar. For years the standard for cider vinegar read:

"The product made by the alcoholic and subsequent acetous fermentation of the juice of apples, is levorotatory, and contains not less than four (4) grams of acetic acid, not less than one and six-tenths (1.6) grams of apple solids, of which not more than fifty (50) per cent are reducing sugars, and not less than twenty-five hundredths (0.25) gram of apple ash in one hundred (100) cubic centimeters (20°C.); and the water-soluble ash from one hundred (100) cubic centimeters (20°C.) of the vinegar contains not less than ten (10) milligrams of phosphoric acid (P_2O_5) and requires not less than thirty (30) cubic centimeters of decinormal acid to neutralize its alkalinity."

As pointed out by Tolman[2] this early standard actually compelled the manufacturer of high-grade cider vinegar to adulterate his product in order to meet the requirements. In later editions there was injected into this definition of cider vinegar the requirement that not more than 50 per cent of the apple solids should be reducing sugars, entirely neglecting the fact that the amount of reducing sugars depended entirely upon the character of the

[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[2] *J. Assoc. Off. Agr. Chem.*, **1939**, 30.

fermentation of the cider. If properly fermented very little sugar was left and a better product obtained, but one frequently containing less than 1.6 per cent of solids.

The standard is now reduced to a simple definition, the question of compliance with the definition being left to the decision of the analyst after studying all data in the light of experience or comparison with known samples. The former inclusion of detailed data was of little material benefit and as a rule served only as a guide to the unscrupulous manufacturer who wished to adulterate his product and conceal the adulteration by showing that the standards were met.

In making comparisons it should be kept in mind that a fundamental basis of the Federal standards is to so set them that a departure of the articles to which they apply, above the maximum or below the minimum prescribed, is evidence that such articles are of inferior or abnormal quality. They are commonly, therefore, decidedly liberal, and it is not at all impossible for manufacturers to prepare a spurious article which will conform to many of the figures given. In the case of vinegar, for instance, Leach and Lythgoe,[1] from the examination of 22 samples of known purity, suggest a standard of purity which is somewhat more rigorous even than the one quoted. They state that pure cider vinegar should contain at least 4.5 per cent of acetic acid and 2 per cent of cider vinegar solids. The ash should constitute at least 6 per cent of the solids. The alkalinity of 1 gram of ash should be equivalent to at least 65 cc. of $0.1N$ acid. The reducing sugars should not exceed 25 per cent of the solids. The polarization, expressed in terms of 200 mm. of undiluted vinegar, should lie between -0.1 and $-4.0°$ Ventzke.

It should be remembered also that certain practices that would otherwise be regarded as adulterations are permissible if they are not of a character deleterious to health and if the sample is correspondingly labeled.[2] Vinegars are allowed to be diluted to standard strength (4 per cent acetic acid), but the label must plainly state the fact. Whether the water is added to the pressed pomace, or later on in the process, makes no difference; the dilution must be indicated on the label. The mixing of vinegars made from different raw materials must be

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[1] *J. Am. Chem. Soc.*, **1904**, 375.
[2] U. S. Dept. Agr., *Food Inspection Decision* **140**.

indicated by the word "compound," and vinegars to which harmless flavors or colors have been added in imitation of some particular variety may be sold if labeled "Imitation Vinegar."

Concerning "second pressings," the number of pressings used in making the juice is held to be immaterial provided the pomace is fresh and not fermented. Pressings from fermented pomace comes under the head of "filthy and decomposed material" and cannot be used at all.

TABLE 73.—CIDER VINEGAR ANALYSES

	Minimum	Maximum	Average
Specific gravity (15.6°C.).............	1.0126	1.0275	1.0177
Acidity as acetic acid, grams per 100 cc.	3.24	9.96	5.21
Non-volatile acid as lactic, grams per 100 cc................................	0.05	0.30	0.18
Alcohol by volume, grams per 100 cc...	0.03	2.00	0.35
Glycerol, grams per 100 cc............	0.23	0.46	0.30
Total solids, grams per 100 cc.........	1.20	4.45	2.40
Reducing sugars as invert sugar, grams per 100 cc........................	0.11	1.12	0.47
Volatile reducing substances as invert sugar, grams per 100 cc............	0.14	0.34	0.20
Non-sugar solids, grams per 100 cc....	1.00	2.90	1.90
Pentosans, grams per 100 cc..........	0.08	0.22	0.16
Formic acid, grams per 100 cc.........	0.0003	0.009	0.003
Volatile esters as ethyl acetate, grams per 100 cc........................	0.30	0.91	....
Total ash, grams per 100 cc..........	0.20	0.57	0.38
Soluble ash, grams per 100 cc.........	0.17	0.51	0.34
Phosphoric acid in soluble ash, grams per 100 cc........................	0.007	0.040	0.017
Phosphoric acid in insoluble ash, grams per 100 cc........................	0.004	0.032	0.012
Alkalinity of soluble ash, cc. $0.1N$ acid.	21.5	56.0	35.0
Polarization in 200-mm. tube, °V......	−0.2	−3.6	−1.4
Sugars in total solids, per cent........	5.3	43.3	20.2
Total ash in non-sugar solids, per cent..	12.0	30.0	19.0

Vinegar made from dried-apple products, skins, cores, and "chops," (by which is meant the whole apple) cannot be called "vinegar" without further designation, but must be plainly marked to show the material from which it is produced. The dried-apple material must, of course, be clean and undecomposed.

In the foregoing table are represented about 100 analyses of pure cask-fermentation and generator-made cider vinegars, before being reduced with water.[1]

Balcom[2] has compiled and calculated to a uniform basis the published results of analyses (about 100) of vinegars of known purity which are of especial interest for the comparison made with several factitious samples of known character. These are shown in the following table:

TABLE 74.—TYPICAL VINEGARS
(Grams per 100 cc.)

| | Total acid | Total solids | Non-sugar solids | Reducing sugars in solids, per cent. | Total ash | Alkalinity of water-soluble ash | Ash in non-sugar solids, per cent. | Phosphoric acid (Mgm. of P_2O_5) | | | Polarization direct, °V |
								Soluble	Insoluble	Total	
Maximum......	7.96	4.52	2.89	45.0	0.52	56.0	26.5	39.9	32.0	64.2	−3.6
Minimum......	3.29	1.37	1.26[1]	5.6	0.20	21.5	11.2	6.7	4.3	15.1	−0.2
Average........	4.94	2.54	1.90	19.6	0.367	35.7	18.8	17.3	12.0	29.3	−1.46
A...............	4.31	0.18	0.16	11.1	0.016	1.5	10.0	0.2	1.5	1.7	+0.6
B...............	4.51	1.27	0.80	37.0	0.20	17.5	25.0	5.5	7.4	12.9	−0.3
C...............	4.72	2.15	1.05	51.2	0.28	37.0	26.7	8.7	10.4	19.1	......
D...............	4.46	2.11	0.91	56.9	0.29	33.0	31.9	11.5	10.8	22.3	−1.0
E...............	4.66	2.09	1.73	17.0	0.56	11.1	32.4	1.8	15.2	17.0	0.0

[1] Abnormally low; the next lowest values are 1.35, 1.35, and 1.39. These four are the only ones out of 63 analyses found to be below 1.40.

The maximum and minimum figures given illustrate very well the important fact that, owing to the variable amounts of sugar in the original juice and the fermented product, the non-sugar solids are much more nearly constant than the total solids, practically never falling below 1.25 per cent. For the same reason, the percentage of ash in the non-sugar solids is a much more valuable factor than the ash in the total solids in determining adulteration.

Sample A is an uncolored spirit or distilled vinegar. Vinegars of this type are frequently colored with caramel and sold as "malt vinegar" or used to adulterate cider vinegar.

B is a mixture of equal volumes of a spirit vinegar A with cider vinegar. In comparison with the average values for cider vinegar, note the lowering in total solids as well as the reversal

[1] BROOKS: "The Legal Chemistry of Food Products."

[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 96.

of the ratio between soluble and insoluble phosphoric acid. The most marked change, however, comes in the lowering of the non-sugar solids.

C is a similar mixture of cider and spirit vinegar, except that boiled cider has been added to bring up the total solids. The adulteration is still shown, however, by the low value of the non-sugar solids, taken in connection with the abnormally high percentage of sugar in the total solids. The relation between soluble and insoluble phosphoric acid remains the same as in sample *B*.

D is a commercial sample, the analysis of which shows it to be of the same nature as *C*. Numerous samples of this kind are on the market and are undoubtedly mixtures of cider vinegar and distilled vinegar, to which has been added some material high in sugar and ash, believed to be in most cases such substances as boiled cider, apple jelly, or unfermented apple juice.

E is a sugar vinegar made from New Orleans molasses by the generator method, a type of vinegar that is being made in increasing quantities to replace the colored spirit vinegar.

The figures show how closely it resembles cider vinegar, the chief difference being in the high ash, low alkalinity of the ash, and very low soluble phosphoric acid.

The following table shows the close correspondence in the usual analytical tests between distilled vinegar and the acetic acid product:

	Distilled vinegar per cent	Artificial vinegar per cent
Total solids............................	0.16–0.3	0.3 –0.45
Ash...................................	0.04–0.09	0.02–0.05
Nitrogen..............................	0.03–0.04	0.00–0.04
Phosphates (P_2O_5)...................	0.02–0.03	0.00–0.03

As a result of an extended investigation into the manufacture of cider vinegar by the generator process, in which over 1,000 samples were analyzed at the factories,[1] the most important determinations for judging the purity of a vinegar were found to be: Total solids, reducing sugars after evaporation, non-sugars,

[1] TOLMAN and GOODNOW: *Ind. Eng. Chem.*, **1913**, 928; BENDER: "Recent Methods for the Detection of Adulterated Vinegar."

ash, acidity, glycerol, and formic acid. It was found that the non-sugars were fairly constant and varied between 1.5 and 2.6 grams per 100 cc.; further, that the percentage of ash in the non-sugars, which is readily calculated when both ash and non-sugars are known, was quite constant, varying between 13 and 19 per cent. The lowest amount of glycerol found in any sample was 0.24 gram per 100 cc., while the highest was 0.40 gram. The highest acetic acid found was 6.3 grams per 100 cc. Commercial acetic acid contains from 0.5 to 1.5 per cent of formic acid; distilled vinegar contains practically none. At present acetic acid, made by modern processes and nearly free from formic acid, is readily available. Better tests are the oxidation value and volatile reducing substances. Cider vinegar, when tested for formic acid by the method described on page

TABLE 75.—ANALYSES OF VINEGAR AND ADULTERANTS
(Grams in 100 cc.)

Determination	1	2	3	4	5	6	7	8
Specific gravity............	1.0520	1.0297	1.0458	1.0133	1.0244	1.0195	1.0212	
Total solids..............	12.94	6.66	5.21	2.02	2.85	2.38	2.87	54.96
Reducing sugar after evap...	8.57	4.42	1.53	0.25	0.57	0.62	0.73	46.94
Polarization, °V,..........	−6.85	−8.38	−7.39	−0.20	−1.12	−1.4	−1.3	−189.6
Ash.....................	0.35	0.19	0.19	0.28	0.33	0.35	0.37	1.38
Soluble ash..............	0.27	0.16	0.19	0.24	0.27	0.29	0.30	1.16
Alk. sol. ash, cc. 0.1N acid..	7.83	13.8	15.3	27.5	33.9	27.2	33.3	88.0
Sol. P_2O_5, mg. in 100 cc.....	11.8	0.84	6.05	12.65	8.04	11.20	11.7	49.0
Insol. P_2O_5, mg. in 100 cc...	13.2	11.8	8.98	9.55	13.9	15.6	24.7	36.0
Total acid...............	0.51	0.21	0.48	4.33	7.87	5.67	6.60	
Pentosans...............	0.23	0.36	0.36	0.14	0.19	0.40	0.36	
Vol. reducing substances....	......	......	......	0.07	0.22	0.31	0.24	

1. Pure apple juice pressed in the laboratory from Baldwin apples.
2. Apple-waste extract made by boiling the skins and cores of the apples used in No. 1.
3. Commercial apple-waste extract.
4. Pure vinegar made in the laboratory from No. 1. Fermentation not carried so far as in commercial products.
5. Pure cider vinegar made by the commercial generator process from russet apples.
6. Second pressings from the stock used in No. 5.
7. Third pressings from the stock used in No. 5.
8. Boiled cider.

432 gives a slight precipitate corresponding to less than .7 mg. of formic acid per 100 cc., due possibly to small amounts of volatile reducing substances retained by the calcium carbonate. The amount found in samples adulterated with commercial acetic acid varies from 15 to 60 mg. per 100 cc.

Table 75 comprises some analyses made in the writer's laboratory of cider vinegar and adulterants from known sources.[1]

The determination of pentosans is of considerable value in showing vinegar made from apple waste or second-pressings cider. In vinegar made from fresh and high-grade material, the pentosans will rarely exceed 0.20 gram in 100 cc., over 0.25 placing the sample under suspicion.

The following illustration of the method of judging adulteration by a critical consideration of the analytical results is given by Bender.[2] It must be kept clearly in mind, however, that the results obtained do not always lead to such clear-cut conclusions as in the ideal case cited here. It does serve, however, to illustrate the method of attacking a problem of this kind. In general it may be said that in a genuine food product conclusions reached by consideration of some of the constituents can usually be checked by the ratio of others. In the case of adulterated or factitious products the comparison will break down much sooner.

Determination	As found	Figured back to glycerol, 0.24
Acid as acetic.............................	4.60	9.20
Solids.....................................	2.04	4.08
Reducing sugars...........................	0.90	1.80
Ash.......................................	0.33	0.66
Ash in non-sugars, per cent...............	28.9	28.9
Glycerol..................................	0.12	0.24
Soluble P_2O_5, mg. per 100 cc.............	12.0	24.0
Insoluble P_2O_5, mg. per 100 cc...........	8.0	16.0
Alkalinity of soluble ash, cc. 0.1N acid......	32.0	64.0
Polarization, °V..........................	−1.2	−2.4
Sugar in solids, per cent.................	44.1	44.1

According to the results as found by analysis and given in the first column, the sample would have to be considered a pure cider vinegar if judged by the early standard on page 438. It does not, however, as can be shown, correspond to the definition incorporated in the present standard that cider vinegar is the fermented product of pure apple juice.

[1] HAMILTON, L. F., Thesis, Mass. Inst. Technol., 1914.
[2] "Recent Methods for the Detection of Adulterated Vinegar."

In the second column, the results have been recalculated from the standpoint of the glycerol, the value of which is found to be 0.12, instead of 0.24, the minimum value reported for pure cider vinegar. This reduction must have come, of course, from dilution with water or some substance containing no glycerol. The acid content of the original vinegar before dilution must, then, have been twice 4.6, or 9.2 grams, in 100 cc. This is an impossible result for cider vinegar and indicates at once that the diluting substance was either distilled vinegar or dilute acetic acid. The ash also is abnormal. A reduction of one-half that of normal vinegar would give about 0.16 gram per 100 instead of the 0.33 that is shown. That the ash has been largely increased by addition is further shown by the fact that nearly 29 per cent of the non-sugars is ash, as compared with the known maximum for normal vinegar of 0.19 per cent.

In the same way the phosphoric acid, calculated back to the original sample, shows 40 mg. per 100 cc., a result considerably in excess of the value for normal vinegar. Valuable information can also be secured from the determination of reducing sugars. The normal value is from 0.3 to 0.8 gram per 100 cc., and in the case under consideration the expected value would be one-half these or 0.15 to 0.4. It is evident, then, that from 0.5 to 0.7 gram of the sugar present has been supplied in some extraneous form, such as boiled cider. From the known composition of the latter material, this would involve adding about 0.12 gram non-sugars. If to this 0.12 we add the 0.17 added ash and subtract the sum from the 1.14 found for the non-sugars, we get the figure 0.85 that represents the true non-sugar content of the original vinegar, a value just about one-half the normal content.

From all these considerations, it can be alleged that the sample is adulterated, in that distilled vinegar or dilute acetic acid, together with foreign mineral matter and some substance high in reducing sugar, has been substituted in large part for cider vinegar. The distinction between distilled vinegar and diluted acetic acid could be carried further by modern methods.

In making comparisons, as above, the values taken should be such as to give the sample the benefit of every doubt. Although the comparison works out nicely from the standpoint of the glycerol content, as illustrated, the calculation can be based on any other determination, in which the result is reasonably

constant for pure cider vinegar and in which none of the substance in question could be introduced by the adulteration, basing the calculation, of course, on minimum values. For example, the determination of acetylmethylcarbinol, page 429, might very well be used in the same way, this value almost never falling below 0.12 gram per 100 cc.

As the methods for the adulteration of vinegar became more refined and exact, in that they are based on more thorough knowledge of the analytical variations of the genuine article, it is evident that the judgment of the purity of a suspected sample cannot be based on any one determination. A fairly detailed analysis is essential.

Selected References

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CHAPTER X

FLAVORING EXTRACTS

The flavoring extracts are, in general, solutions in alcohol of the proper strength of the odorous principles obtained from aromatic plants. They may be prepared by direct maceration in alcohol of the desired portion of the plant, as in the case of vanilla; or by simple solution in alcohol of the essential oil obtained from the plant by distillation or pressure, as in the commercial methods for making lemon extract. They are of considerable interest to the food analyst because of the different analytical methods that their examination involves, and because of the ingenious substitutes and forms of adulteration that their relatively high price causes.

Three typical examples will be considered: vanilla, lemon, and ginger. ·

EXTRACT OF VANILLA

Vanilla extract is prepared from the fruit of the *Vanilla planifolia*, a climbing plant of the orchid family and a native of Mexico, from which country the most highly prized vanilla is still obtained. The dried and cured fruits, or so-called "vanilla beans," as they appear in commerce, are long and slender, of a lustrous brown color, and often covered with fine needle-like crystals of vanillin.

Besides the Mexican beans, other and important varieties are the Bourbon (from the island of Réunion), Seychelles, Comores, Madagascar, and Tahiti. The last-named variety is of inferior quality and yields a low-grade extract, being used chiefly for blending and "body." The Tahiti vanilla brings much the lowest price in the market, selling for less than $1 a pound, while the best Mexican varieties will bring from $5 to $8.

Preparation of Vanilla Extract.—The customary method of preparing the extract is by maceration of the chopped beans in alcohol of about 50 per cent strength. Sugar is sometimes added

both to sweeten the product and by osmosis to assist the extraction. Some manufacturers use glycerol for the same purpose.

A typical formula, and one that has served as a basis for many commercial processes, is that formerly given in the U. S. Pharmacopœia[1] for the preparation of "Tincture of vanilla":

Vanilla, cut into small pieces and bruised, 100 grams; sugar, in coarse powder, 200 grams; and alcohol and water, each in sufficient quantity to make 1,000 cc.

Mix 650 cc. of alcohol with 350 cc. of water; macerate the vanilla in 500 cc. of the mixture for 12 hours; then drain off the liquid and set it aside. Transfer the vanilla to a mortar, beat it with the sugar into a uniform powder, then pack it in a percolator, and pour upon it the reserved liquid. When this has disappeared from the surface, continue the percolation by gradually pouring on sufficient menstruum to make 1,000 cc. of tincture.

Many manufacturers use a more dilute alcohol than is prescribed in the method of the Pharmacopœia, although a strength less than 45 per cent is impracticable on account of yielding a gelatinous or gummy extract. The time of extraction is usually much longer, being extended in many cases to several months, in order to exhaust the bean and especially to remove the color, which is slow of extraction. Occasionally, potassium bicarbonate may be added to assist in dissolving the fragrant resin and thus economize in alcohol.

Forms of Adulteration.—The adulterations of vanilla extract may consist in the substitution of some other natural extract that has a flavor resembling that of vanilla or in the use of an entirely factitious product so prepared as to resemble in appearance and in odor the genuine article. Typical of the first form are mixtures having as their base extract of tonka bean, prepared from the seeds of a tropical tree, *Coumarouna odorata*. This extract resembles true vanilla in its general appearance and has a somewhat similar though coarser odor. Artificial extracts are usually made up of weak alcohol containing synthetic vanillin and coumarin, the active principles of vanilla and tonka, respectively (see page 451), sometimes with an addition of prune juice or similar material to give more body to the preparation. Caramel is used for color. Although they cost only a fraction of a

[1] Eighth Revision, p. 484.

true extract they fail to match its delicate flavor, which is modified and perfected by minor constituents, such as gums and resins, as important as the vanillin content.

Weak tinctures of true vanilla, obtained by macerating again the residues from the preparation of high-grade extracts, are sometimes used as a basis for adulterated extracts. These are usually reinforced or strengthened by the addition of synthetic vanillin or coumarin, or both, and colored with caramel or occasionally coal-tar colors.

METHODS OF ANALYSIS

Total Solids.—Use 10 cc. of the sample and proceed as directed on page 312, *a*.

Ash Data.—Determine the total, water-soluble and water-insoluble ash, and the alkalinity of the soluble and insoluble ash as described under General Methods, page 23 *et seq.* Calculate the ash as grams per 100 cc. of extract.

Acidity.—Dilute 10 cc. of the extract to 200 cc. and titrate with $0.1N$ alkali, using phenolphthalein as indicator.

Alcohol.—Measure out 25 cc. of the sample, add 100 cc. of water, and distill 100 cc. as directed on page 486. The distillate may be tested for the presence of methyl alcohol, if desired, by the methods described on page 508 *et seq.*

Vanillin and Coumarin. *Gravimetric Method.*[1]—Measure 50 cc. of the extract into a 250-cc. beaker with marks showing volumes of 80 cc. and 50 cc.; dilute to 80 cc. and evaporate to 50 cc. in a water bath kept at 70°C. or lower. Dilute again to 80 cc. and evaporate to 50 cc. Transfer to a 100-cc. flask, rinsing the beaker with hot water, add from a pipette 25 cc. of neutral lead acetate solution (80 grams of pure crystallized lead acetate per liter), make up to the mark with water, shake and allow to stand 18 hours at 37 to 40°C. in a bacteriological incubator, water bath provided with a thermostat, or other suitable apparatus. If it is desired to determine the lead number on the same sample, a blank with 25 cc. of the lead acetate, made up to 100 cc. with water, should be run under the same conditions. Filter through a small dry filter and measure 50 cc. of the filtrate

[1] HESS and PRESCOTT: *J. Am. Chem. Soc.*, **1899,** 256; WINTON and others: *J. Am. Chem. Soc.*, **1902,** 1128; **1905,** 719; U. S. Dept. Agr., *Bur. Chem. Bull.* **132,** p. 109; *Bull.* **137,** p. 120.

into a separatory funnel. Save the remainder of the filtrate for the determination of lead number as described on page 457.

To the portion in the separatory funnel add 20 cc. of ether[1] and shake. Draw off carefully the aqueous liquid together with any ether emulsion that may have formed, and transfer the clear ether solution to another separatory funnel. Shake the aqueous liquid three times more with ether in the same manner, using 15 cc. each time.

Shake the combined ether extracts four or five times with ammonium hydroxide (1 part of strong ammonia to 11 parts of water), using 10 cc. for the first shaking and 5 cc. for each subsequent shaking. In drawing off the ammoniacal solution, take care that none of the ether solution passes along with it. Reserve the ether solution for the determination of coumarin.

Add to the ammoniacal solution 10 per cent hydrochloric acid to slightly acid reaction. This should be done without delay, since the ammoniacal solution on standing grows darker slowly with a loss of vanillin. Cool and shake out in a separatory funnel with four 10-cc. portions of ether, as described for the first ether extraction. Evaporate the ether solution at room temperature in a weighed dish, dry over sulphuric acid, and weigh. (The vanillin often appears first in the form of oily droplets, which on standing crystallize into light-colored masses.) The residue should be pure vanillin, free from any appreciable amount of color and with a melting point of 80°C.

Transfer the ether solution to a weighed dish and allow the ether to evaporate at room temperature. Dry in a sulphuric acid desiccator and weigh. If the residue is coumarin it will be recognized by its characteristic odor resembling "sweet grass," and if not perfectly white should be purified by treating with three or four portions of petroleum ether (boiling point 30 to 40°C.). Stir with each portion for 15 minutes, decant carefully; finally dry and reweigh the dish, taking the coumarin as the loss in weight. Evaporate the petroleum ether in a porcelain dish and confirm the presence of coumarin by Leach's test (see Notes, p. 452).

Notes.—The method given for the separation of vanillin and coumarin is based on the difference in their chemical constitu-

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[1] For all extractions of the vanillin or coumarin use ether that has been washed twice with water to remove alcohol.

tion. Vanillin, *m*-methoxy-*p*-oxybenzaldehyde, is found in the vanilla bean up to 3 per cent, but is now made artificially on a considerable scale by the oxidation of coniferin or of the eugenol of oil of cloves with alkaline potassium permanganate, from guaiacol by hydrolysis, or other processes. Coumarin is the anhydride of coumaric acid, and may also be prepared synthetically from salicylaldehyde and acetic anhydride. On account of the aldehydic nature of the vanillin compound, the separation by dilute ammonia is possible, the aldehyde-ammonia compound of vanillin being readily soluble in water while the coumarin remains wholly in the ether.

The method as described is not applicable to concentrated or reinforced vanillin and coumarin preparations in which the quantity of vanillin and coumarin present in 50 cc. exceeds the amount soluble in 100 cc. of water at 20°C. In such cases use a smaller sample and dilute to 50 cc.

Although the method as outlined ·should theoretically give pure residues of vanillin and coumarin, this is seldom the case, the residues, except with entirely artificial extracts, being contaminated with gummy or resinous matter. The results obtained by weighing the residues directly, without purification, are consequently usually too high, the error sometimes amounting to 0.04 per cent. If the weighed residues of vanillin and coumarin are discolored, indicating impurities, they should be purified as described above under the determination of coumarin, except that in the case of vanillin the treatment with low-boiling (below 40°C.) petroleum ether should be more prolonged, not less than 15 extractions being made with the boiling solvent. This repeated treatment is necessary on account of the gummy character of the impurities, which prevents the ready solution of vanillin or coumarin with which they are intimately mixed. The proximity of a flame should, of course, be carefully avoided during this operation.

A simpler and less tedious method of purifying the residues is by sublimation, as suggested by Hiltner.[1] To do this, heat the residue, which has been dried over sulphuric acid and weighed, in an oven at 105°C. for 1 or 2 hours, or until its weight is constant. The loss in weight is vanillin or coumarin, as the case may be. If it be desired to test the purity of the volatile matter,

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **152**, p. 135.

the dish may be heated cautiously at first on a hot plate, the sublimate condensed on a cool watch glass and examined by appropriate tests. The dish and residue should then be placed in the oven as above, in order to complete the volatilization. Vanillin and coumarin volatilize at 105°, leaving the gummy matter unchanged. Wichmann[1] has shown that pure vanillin leaves a slight residue at 105°C., but the error caused is negligible. The purity of the vanillin may also be determined by the colorimetric method (page 453).

The residue, or the sublimate obtained in the volatilization method, should be subjected to qualitative tests in order to show that it is vanillin or coumarin. In the case of the vanillin, a small amount of the residue, dissolved in 2 drops of concentrated hydrochloric acid, should give a pink color upon the addition of a crystal of resorcin.

Also a portion of the vanillin dissolved in 2 or 3 drops of ether and allowed to evaporate spontaneously on a microscope slide should show a characteristic appearance with polarized light. The vanillin crystallizes in slender needles, forming star-shaped clusters or sphaerulites. These give a brilliant play of colors owing to their high birefringence, with crossed Nicols.

The coumarin residue should have the characteristic "sweet grass" odor of coumarin, and give a positive reaction with iodine.[2] To make the iodine test, add a few drops of water to the residue, warm gently and transfer to a white porcelain dish or spot plate. Add a few drops of a solution of iodine in potassium iodide, and stir with a glass rod. If coumarin is present, a brown precipitate will form and gradually collect in dark-green clots. Care should be taken that the solutions are not too dilute, since the test is not one of extreme delicacy.

If vanillin only is to be determined it will probably be found a saving of time to employ the colorimetric method given below; or the original extract may be tested for coumarin by Wichmann's method (page 455), and if this is found to be absent, the determination of vanillin may be simplified by evaporating and weighing the ether extract without treating it with ammonia and hydrochloric acid.

[1] *J. Assoc. Off. Agr. Chem.*, **1921**, 481.
[2] Leach-Winton: "Food Inspection and Analysis," 4th ed., p. 923.

If coumarin only is to be determined the following method[1] will be found satisfactory: To 50 cc. of vanilla extract add lead acetate in slight excess, make up to 100 cc., filter, and remove the excess of lead from the filtrate by dry potassium oxalate. Extract 50 cc. of the deleaded solution with three or four portions of ether, add to the ether solution a few drops of phenolphthalein solution and a slight excess of alcoholic potash. Remove the vanillin salt by washing with several 10-cc. portions of water until the disappearance of the red phenolphthalein color in the wash water. Evaporate the washed solutions, dry and weigh the coumarin.

Colorimetric Determination of Vanillin.[2] *Reagent.*—To 100 grams of pure sodium tungstate and 20 grams of phosphomolybdic acid (free from nitrates and ammonium salts) add 100 grams of sirupy phosphoric acid, (containing 85 per cent H_3PO_4), and 700 cc. of water; boil over a free flame for $1\frac{1}{2}$ to 3 hours; then cool, filter if necessary, and make up with water to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

Process.—Measure 5 cc., or a sufficient amount to furnish 8 to 12 mg. of vanillin, of the vanilla extract with a pipette into a 100-cc. flask, add 75 cc. of water, 4 cc. of lead acetate solution, containing 5 per cent basic and 5 per cent neutral lead acetate, (see Notes), and make up to 100 cc.

Filter rapidly through a fluted filter paper and transfer 5 cc. of the filtrate with a pipette to a 50-cc. flask. In another 50-cc. flask place 5 cc. of the standard vanillin solution (0.1 gram per liter); then pipette 5 cc. of the phosphotungstic-phosphomolybdic reagent into each flask, allowing it to run down the neck of the flask in order that any adhering vanillin may be washed down. After shaking, allow the flask to stand for 5 minutes and then fill to the mark with 20 per cent sodium carbonate solution. Invert the flask several times to mix the contents, and allow to stand for 15 to 20 minutes for complete precipitation of the sodium phosphate. Filter rapidly through fluted filters and compare the color of the resultant deep-blue solutions in a Duboscq or other colorimeter. The standard solution is best

[1] WICHMANN: *Ind. Eng. Chem.*, **1918**, 537.

[2] FOLIN and DENIS: *Ind. Eng. Chem.*, **1912**, 670; CURL and NELSON: *J. Assoc. Off. Agr. Chem.*, **1939**, 684.

placed at 20 mm. if using the Duboscq instrument, as experience has shown that the best reading is obtained at this point. Do not read the solutions unless they are absolutely clear. The readings should be made without any great delay, since the color darkens slowly for about 1 hour after adding the alkali. After 30 minutes the readings are worthless.

Notes.—It has been shown by Curl and Nelson that the ratio of neutral to basic lead acetate in the clarifying agent is important. Too large a proportion of the neutral salt is unsatisfactory, especially in vanillas of high resin content, because it fails to precipitate interfering substances and the results are too high. On the other hand, too low a ratio of neutral to basic lead acetate carries down vanillin and gives low results. The ratio of neutral to basic lead acetate should not be much greater or less than 3 to 1, and in preparing the solution it is really necessary to analyze the basic lead acetate and make certain that the reagent as finally used meets this condition.

The standard vanillin solution should be reasonably freshly prepared, since on standing at room temperature it tends to oxidize to vanillic acid which gives a deeper color with the Folin reagent. The solution retains its correct value better if the bottles are kept filled and in the refrigerator.

Vanillin and other mono-, di-, and trihydric phenolic compounds give the deep-blue color with Folin's reagent[1] and excess of sodium carbonate. Many substances interfere, including aromatic amines, many sugars, and alkaloids. Tannin and tannic acid interfere by reducing the reagent, but are removed by lead acetate.

Before taking readings, the correctness of the colorimeter should be tested by placing the standard solution in both tubes and noting the readings, which should agree within 1 per cent. A slight disagreement may sometimes be corrected by varying somewhat the relative position of the instrument and the source of light (see also page 29). Readings should not be taken as final if the variation between the standard and the unknown is greater than 20 per cent, but in such a case the determination should be repeated, using a fresh standard and a proportionately greater or less amount of the filtrate from the lead precipitate.

[1] *J. Biol. Chem.*, **1927**, 627–650.

The amounts of reagents should be added carefully, since variation in this respect sometimes causes varying results.

Apart from serving as a direct determination of the vanillin, the colorimetric method may be used as a check on the gravimetric method or to replace the tedious process of purification. To do this dissolve the impure vanillin residue (page 450), after drying and weighing, in the smallest possible quantity of 25 per cent alcohol, transfer to a graduated flask, and make up with water to such a concentration that 10 cc. of the solution will contain approximately 1 mg. of the residue. To 5 cc. of this solution, add 5 cc. of the phosphotungstic-phosphomolybdic reagent, as described above, and compare with a standard prepared in the same way. No clarifier will be needed.

In the experience of the writer and others the colorimetric method has been found satisfactory for standard extracts but not reliable for many of the colored, highly reinforced extracts on the market for the baking and confectionery trade.

Wichmann Test for Coumarin.[1]—To 10 cc. of the extract add 10 per cent sodium hydroxide solution until alkaline, then dilute with 15 cc. of water to reduce the alcoholic strength and extract with 20 cc. of ether in a separatory funnel. The ether solution will be slightly colored when the brown lower layer has been drawn off. Add a few cubic centimeters of strong alcoholic potash, shake the mixture, and wash with 10 cc. of water. The ether layer will then be found to be white. This procedure removes all organic acids, vanillin, coloring matter, or saccharin that might be present. Place 1 cc. of 50 per cent potassium hydroxide solution in a test tube, pour the ether solution of coumarin over it, shake thoroughly and quickly evaporate the ether on a steam bath. Then place the tube over a free flame, evaporate the water, and fuse the potassium hydroxide. If coumarin is present in any amount a change of color will be noticed as the evaporation of the water proceeds and fusion begins. Even very small quantities of coumarin in strong, hot potassium hydroxide solution will show a greenish-yellow color that suddenly disappears as the heating is continued. The disappearance of the color shows that the coumarin has been converted into the salicylate and heating should be discontinued. Take up the melt with a few cubic centimeters of water, acidify

[1] *Ind. Eng. Chem.,* **1918,** 536.

with sulphuric acid, and extract in a small separatory funnel with 5 to 10 cc. of benzene. Remove the acid solution from the funnel and wash the benzene with a few cubic centimeters of water. After washing, filter the benzene into a test tube and test for salicylic acid with 1 or 2 cc. of water containing a few drops of ferric chloride solution. If no color develops on shaking, one or two drops of 0.1N sodium hydroxide should be added to neutralize any trace of mineral acid that may be present and prevent the development of the purple color.

Notes.—When coumarin,

$$CH{=}CH$$

$$O{-}C{=}O$$

, is fused with potassium hydroxide, potassium salicylate,

$$COOK$$
$$OH$$

, is formed.

. Vanillin,

$$CHO$$
$$OCH_3$$
$$OH$$

, under the same conditions forms potassium protocatechuate,

$$COOK$$
$$OH$$
$$OH$$

.

Benzene is preferable as a solvent because of its low density, low solvent power for mineral acids, and because it will not dissolve any protocatechuic acid formed from vanillin that might possibly have been carried over with the ether.

The change of color on fusion indicates its own end point and gives, together with the purple salicylate color, a double test for coumarin. Coumarin is changed to the salt of coumaric acid by hot concentrated potassium hydroxide. The development of the yellow color shows this phase, and the sudden disappearance of the color indicates the conversion into a colorless salicylate.

The test is not quantitative because of the slight volatilization of the coumarin before the alkali can react with it.

Lead Number. *a. Normal Lead Number.* (*Winton*[1]).—Mix 10 cc. of the filtrate from the lead acetate precipitate obtained in the determination of vanillin and coumarin (page 450) with 25 cc. of recently boiled distilled water and a moderate excess of sulphuric acid. Add 100 cc. of 95 per cent alcohol and mix again. Let stand overnight, filter on a Gooch crucible, wash with 95 per cent alcohol, dry in the water oven, ignite at low redness for 3 minutes, taking care to avoid the reducing flame, and weigh. Run a blank under exactly the same conditions in order to determine the value of the lead acetate and calculate the result by the following formula:

$$P = \frac{100 \times 0.6831(S - W)}{5},$$

where P = normal lead number, S = grams of lead sulphate corresponding to 2.5 cc. of the lead acetate as determined in the blank analysis, and W = grams of lead sulphate obtained in 10 cc. of the filtrate from the lead acetate precipitate as described.

Notes.—If no suitable apparatus is available for keeping the solutions at 37 to 40°C., they may be allowed to stand overnight at room temperature. Winton and Berry found, however, that the amount of lead precipitated is materially affected by the time of standing after adding the lead acetate solution, and by the temperature of the solution while standing. The temperature of 37°C. was chosen because bacteriological incubators, kept at that temperature, are available in many laboratories.

The method is an adaptation of the one devised by Winton for determining the purity of maple products (see page 320). The use of basic lead acetate, however, is objectionable in the case of vanilla extract, since it carries down vanillin, thus precluding the possibility of determining this in the same sample. Neutral lead acetate is free from this objection.

If desired, the lead may be determined as chromate instead of as sulphate, with the advantages of not having to stand overnight and saving of alcohol reagent.

[1] WINTON and LOTT: U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 110; WINTON and BERRY: U. S. Dept. Agr., *Bur. Chem. Bull.* **152**, p. 148.

Method.—Pipette 10 cc. of the clear filtrate from the lead ace-
tate precipitate into a 400-cc. beaker, add 2 cc. of glacial acetic
acid, 25 cc. of water, and 25 cc. of potassium bichromate (5 grams
per liter).   Heat the beaker and contents immediately with a
moderate flame and continue heating until the precipitate changes
in color from yellow to orange.   Then filter the solution through a
weighed Gooch crucible provided with an asbestos mat and wash
thoroughly with hot water and then with a few cubic centimeters
each of alcohol and ether.   Dry at 100°C., cool in a desiccator,
and weigh.   Determine the lead in the blank in the same way.
Calculate the lead number as above, substituting the weights of
lead chromate for lead sulphate in the formula, and using the
factor 0.6411.

The determination will be facilitated by observance of the
following precautions: Do not put a stirring rod into the solution
while boiling.   Do not heat the solution before adding potassium
bichromate or allow the mixture to stand after adding the potas-
sium bichromate.   By the time the solution has come to boiling,
the precipitate will have changed to an orange red.   When trans-
ferring the bulk of the solution to the Gooch crucible, use the
stirring rod sparingly.   If the precipitate comes in contact with
the rubber policeman, wash it with hot water as soon as possible.
Use the policeman vigorously in removing the precipitate from
the sides of the beaker.   The precipitate that adheres to the
policeman can be removed by rubbing it vigorously against the
sides of the beaker with an abundance of hot water.   The filtrate
must contain an excess of potassium bichromate; if it does not,
increase the concentration or the volume of the potassium bichro-
mate used.

b. Lead Number—(*Wichmann*).[1]—If it is not desired to deter-
mine the vanillin and coumarin on the same sample as the lead
number, or if pressed for time, the following modification of the
method will be found advantageous.

Procedure.—Place 175 cc. of boiled distilled water in a round-
bottomed flask of 1-liter capacity.   Add by means of pipettes
25 cc. of lead acetate solution[2] and 50 cc. of the sample.   Place

[1] *Ind. Eng. Chem.*, **1921**, 414.
[2] Dissolve 80 grams of neutral lead acetate in distilled water that has been
recently boiled, dilute to 1 liter, and filter if the solution is not clear.

the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. The hole in the board should be of such size that, when the flask contains 50 cc. of liquid, the level of the liquid will be even with the top of the board, or slightly above it. Connect the flask to a condenser, and with a moderate flame distill 200 cc. into a volumetric flask. The approximate alcohol content of the extract may be calculated from the specific gravity of the distillate if desired. Transfer the residual solution to a 100-cc. volumetric flask by means of carbon dioxide-free water and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc. with carbon dioxide-free water, mix, and filter through a dry filter.

In 10 cc. of the filtrate determine the lead as sulphate or chromate as described on pages 457 and 458.

For the blank determination proceed as before, but use 5 drops of glacial acetic acid instead of the sample and distill 150 cc. instead of 200 cc.

Notes.—In the above method no dealcoholization at low temperature or long standing is required. When the precipitation is made at boiling temperature an equilibrium is quickly established, and colloidal extracts, notably those made from Tahiti beans, which give much trouble in the older method, can be filtered without difficulty.

The values obtained by the Wichmann modification run somewhat higher than those by the older method, averaging from one-third to one-half more, the greater differences coming with the colloidal extracts.

High lead numbers will result by either method in the case of extracts reinforced with vanillin, especially with diluted extracts thus strengthened, on account of the precipitation of an insoluble lead-vanillin compound. In case, then, of abnormally high vanillin content, the method should be modified to permit the extraction of this with ether before determining the lead number.

Detection of Caramel.—The test for caramel depending upon its insolubility in paraldehyde, as described on page 98, may be used to show its presence in vanilla extracts if preceded by the treatment with zinc hydroxide. Positive results should, however, be interpreted with caution as indicating the presence of

caramel, because with some low-grade natural extracts, especially those made from Tahiti beans, some resinous extractive matter is thrown down by paraldehyde, giving a flocculent precipitate. A positive result should not be declared unless a dark-brown precipitate, adherent to the walls of the tube, is obtained. Unless considerable experience has been had with the paraldehyde test, better results will probably be obtained with the methods described below, especially the first one.

1. Marsh Test.[1]—Evaporate 25 cc. of the extract just to dryness on the steam bath or until the alcohol has been removed. Dilute the remainder with water and alcohol, using 13.0 cc. of 95 per cent alcohol (equivalent to 12.5 cc. of absolute alcohol) and making up to the mark in a 50-cc. flask with water. Transfer 25 cc. of this solution to a separatory funnel, add 25 cc. of the freshly shaken Marsh reagent, and shake, not too vigorously to avoid emulsification. Allow the layers to separate but still to remain in the funnel, and repeat the shaking twice more. After the layers have separated clearly, draw off the lower layer into a 25-cc. cylinder, and make up to volume with 25 per cent (by volume) alcohol. Compare in a colorimeter with the remaining 25-cc. portion (which has not been extracted with the reagent), filtering both solutions before placing in the colorimeter. Express the result as *per cent of color insoluble in amyl alcohol.*

The Marsh reagent is prepared as follows: Mix 100 cc. of amyl alcohol, 3 cc. of sirupy phosphoric acid, and 3 cc. of water; shake before using. If the reagent becomes colored on standing, the amyl alcohol should be redistilled over 5 per cent phosphoric acid.

Note.—The method is based on the fact that the natural coloring matter of the vanilla bean is largely soluble in acid amyl alcohol, while caramel is almost completely insoluble. Hence, in caramel-colored extracts, there will generally be a preponderance of color insoluble in amyl alcohol; whereas in genuine vanilla the proportion of insoluble color rarely exceeds 35 per cent and is usually below 25 per cent. See page 576 for the application of the method to detect artificial color in whisky.

2. Lead Acetate Test.—Evaporate a portion of the sample to about one-third of its volume to remove alcohol, dilute to the original volume with water, add an excess of neutral lead acetate, and filter. If caramel is present the filtrate will be deep yellow-

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **152**, p. 149.

brown to brown; if the extract is pure the filtrate will be colorless to pale yellow.

To those experienced in the examination of vanilla extracts, the general appearance of the lead filtrate will serve to distinguish pure extracts from those colored with caramel.

Vanilla Resins. *Procedure.*—Pipette 50 cc. of the extract into a small beaker; add 50 cc. of water; evaporate to 50 cc. on a steam bath; add 50 cc. of water; and again evaporate to 50 cc. Cool. If the mixture has an acid reaction, add 2 cc. of dilute hydrochloric acid $(1 + 1)$. If the mixture is not acid to litmus, add dilute hydrochloric acid $(1 + 1)$, drop by drop, until distinctly acid to litmus paper, then 1 cc. in excess. Cover and let stand overnight. Filter; wash six or seven times with approximately 0.05 N hydrochloric acid [9 cc. hydrochloric acid $(1 + 1)$ per liter of water]. Dissolve the resin in warm 95 per cent alcohol by pouring through the filter. Evaporate the alcohol in a weighed 50-cc. beaker and dry to constant weight at 100°C. Report results to two decimal places only. Reserve the resin for qualitative tests.

Notes.—The determination of the approximate amount of resins present, as is true with the lead number, affords a general idea of the proportion of true vanilla present in an extract. The value obtained should not be used to calculate with literal exactness the per cent of vanilla, because the results are not strictly proportional to increasing dilution.

The normal quantity of resin in a standard extract, as determined by the above method, is about 0.08 to 0.11 gram per 100 cc.

It may be advisable also to determine the character of the resin by appropriate qualitative tests. For these consult "Official Methods of Analysis," **1935,** page 308, of the Association of Official Agricultural Chemists, or Sale and Goodrich.[1]

Coal-tar Colors.—These, if present, can be detected by dyeing on wool and identified by the tests described in the chapter on Colors, pages 70 to 94.

Simple Tests to Distinguish Artificial Extracts.—To distinguish between genuine extracts and artificial imitations, of which there are many on the market, is comparatively easy, and several simple qualitative tests may be sufficient to identify a sample.

[1] *J. Assoc. Off. Agr. Chem.*, **1926,** 451.

To one at all experienced in the examination of vanilla extract, the odor of coumarin is easily apparent in a sample containing it, even without extraction, and needs no further evidence to show its presence.

The behavior on evaporation is of distinct value. If a portion of the sample be evaporated to half its volume on the water bath, and on dilution to the original volume remains as clear as it was at first, it can contain very little true vanilla extract, but is probably entirely artificial. Unless the resins of true vanilla are held in solution by potassium bicarbonate or ammonia, as is sometimes the case, they will precipitate when the alcohol is evaporated and give a turbid solution. Artificial vanilla, on the other hand, having no resin to precipitate, remains clear.

Extracts that are entirely artificial may also be shown by the following simple test: Shake 5 cc. of the extract in a test tube with an equal quantity of ether and allow the layers to separate. On another portion make a similar test, using amyl alcohol instead of ether. With genuine extracts the ether and amyl alcohol, respectively, will be colored from various shades of yellow to brown owing to the natural color and resin; with artificial extracts, usually colored with caramel, the ether and amyl alcohol will be colorless. It should be stated that the presence of color in the amyl alcohol or ether does not necessarily mean that the extract is pure, since it may be due to coal-tar colors or to extractive matter other than vanilla resin, but on the other hand the absence of color indicates the absence of vanilla. With such samples the use of the word "vanilla" on the label, without any qualifying term to show that it is an imitation, is illegal, since no appreciable quantity of true vanilla can be present.

INTERPRETATION OF RESULTS

It would naturally be expected that vanilla extract would be of varying composition because of differences in the grades and varieties of vanilla bean from which it is made, as well as variations in the solvent employed and the length of time during which maceration in the solvent is allowed to continue. With all these variations, however, the extract of true vanilla has certain pronounced characteristics that serve to distinguish it easily from artificial imitations and fairly readily from extracts made from somewhat similar natural products.

Presumably, because of these considerable variations in raw materials and manufacturing processes, the Federal standard[1] is left exceedingly vague and indefinite, merely requiring that vanilla extract shall be prepared from the dried, cured fruit of *Vanilla planifolia, Vanilla fragrans*, with or without sugar, dextrose or glycerol, and shall contain in 100 cc. the soluble matters from not less than 10 grams of the vanilla bean.

A better basis for detecting adulterants will be obtained by comparing directly analyses of genuine and adulterated extracts.

Composition of Genuine Vanilla Extract.—The most enlightening series of analyses of genuine vanilla extract is that made by Winton, Albright and Berry[2] of 75 samples prepared in the laboratory from all the standard varieties of the vanilla bean. The extracts were made by the process of the U. S. Pharmacopœia (see page 448) which is probably less efficient than some of the commercial methods. This, however, is by no means an injustice to the manufacturer, since any standard based on analyses of U. S. P. extracts would be rather favorable toward a commercial extract prepared by a more thorough method of extraction. A summary of these analyses is given in Table 76.

Some of the more important results obtained by the same methods on pure commercial extracts, made from various blends of vanilla beans by commercial methods rather than the U. S. P. process, are summarized in Table 77.

These extracts were obtained[3] from reputable manufacturers, who guaranteed their purity and furnished an outline of the formulas by which they were made, as stated below:

No. 1. Extract made from the best grade of Mexican beans, with a menstruum of sugar sirup, 40 per cent "cologne spirits" and 1 per cent glycerol; macerated for 2 months and then percolated. The finished product was adjusted to accord with the U. S. standard, as given above.

No. 2. Extract made from average quality Bourbon beans with sugar and 50 per cent alcohol; macerated for 16 months and then percolated. The finished product was diluted so as

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[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **152**, p. 146; *Ind. Eng. Chem.*, **1915**, 516.

[3] HILTNER: U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, p. 82.

to contain the extracted matter of 1 pound of beans in 1 gallon of the extract. (In terms of the U. S. standard this is equivalent to about 12 grams per 100 cc.)

TABLE 76.—COMPOSITION OF PURE VANILLA EXTRACTS

Kind of bean	Vanillin grams in 100 cc.	Normal lead number	Acidity, cc. 0.1N alkali per 100 cc.	Ash, grams in 100 cc.			Alkalinity of ash, cc. 0.1N acid per 100 cc.		Color insoluble in amyl alcohol, per cent
				Total	Water-soluble	Water-in-soluble	Water-soluble	Water-in-soluble	
Mexican:									
Maximum..	0.20	0.68	52	0.422	0.349	0.073	40	13	24.4
Minimum..	0.15	0.47	42	0.297	0.246	0.037	29	7	19.0
Average.....	0.17	0.58	46	0.359	0.301	0.058	35	10	21.2
Bourbon:									
Maximum..	0.22	0.63	51	0.373	0.319	0.080	34	18	30.3
Minimum..	0.13	0.44	35	0.263	0.220	0.043	25	9	21.3
Average....	0.18	0.52	40	0.317	0.259	0.058	27	13	26.6
Seychelles:									
Maximum..	0.21	0.60	42	0.316	0.262	0.058	30	17	29.4
Minimum..	0.16	0.45	35	0.251	0.213	0.038	24	9	22.7
Average....	0.19	0.51	39	0.293	0.243	0.050	27	12	25.6
Madagascar:									
Maximum..	0.30	0.63	47	0.326	0.271	0.060	33	14	30.3
Minimum..	0.16	0.40	42	0.220	0.193	0.027	26	8	23.2
Average....	0.22	0.50	45	0.284	0.239	0.045	28	11	26.8
Comores:									
Maximum..	0.31	0.74	47	0.432	0.357	0.081	38	17	30.3
Minimum..	0.12	0.40	34	0.229	0.182	0.037	22	9	20.4
Average....	0.18	0.59	40	0.333	0.272	0.061	31	14	26.7
South American:									
Maximum..	0.23	0.58	52	0.344	0.295	0.054	30	12	29.4
Minimum..	0.19	0.49	44	0.305	0.261	0.044	26	12	20.0
Average....	0.21	0.52	49	0.325	0.276	0.049	28	12	23.3
Java:									
Maximum..	0.24	0.61	52	0.349	0.299	0.050	38	11	35.7
Minimum..	0.22	0.44	45	0.290	0.246	0.044	31	7	32.2
Average....	0.23	0.50	48	0.311	0.265	0.046	34	8	34.5
Tahiti:									
Maximum..	0.17	0.56	33	0.288	0.249	0.042	29	8	22.0
Minimum..	0.11	0.44	30	0.221	0.179	0.039	23	7	16.0
Average....	0.13	0.50	31	0.254	0.214	0.040	26	7	18.0
All Analyses:									
Maximum..	0.31	0.74	52	0.432	0.357	0.081	40	18	35.7
Minimum..	0.11	0.40	30	0.220	0.179	0.027	22	7	16.0
General Average......	0.19	0.54	42	0.319	0.265	0.054	30	12	25.5

No. 3. Extract made from Tahiti beans of average quality by the so-called "machine process" at a temperature of 100°F.,

requiring only about 36 hours to complete the manufacture. The menstruum was similar to No. 2. The product was diluted to about U. S. standard strength.

TABLE 77.—COMPOSITION OF PURE COMMERCIAL VANILLA EXTRACTS

Sample	Vanillin, per cent	Normal lead number	Color insoluble in amyl alcohol, per cent
1	0.20	0.50	25.6
2	0.34	0.55	22.8
3	0.19	0.50	13.3
4	0.18	0.45	17.4

No. 4. Extract made according to the following formula: 29 lb. of Tahiti beans, 12 lb. of prime Bourbon beans, menstruum 49 gal. of sugar and 60 per cent alcohol; macerated for about 1 month, percolated, and finally diluted with water to 50 gal., approximately equivalent to the U. S. standard.

Standards of Purity.—From the results obtained in the studies of pure vanilla extracts referred to above, the following limiting values have been suggested for genuine vanilla extract:

Vanillin, not less than 0.10 nor more than 0.35 per cent.

Neutral lead number, not less than 0.40 nor more than 0.80.

Total ash, not less than 0.20 nor more than 0.43 per cent.

Acidity, not less than 28.

Color insoluble in amyl alcohol, not more than 35 per cent. (25 per cent is rarely exceeded).

Detection of Adulteration.—True extract of vanilla is characterized by its content of vanillin, varying within the limits assigned above; the presence of resins, the formation with neutral lead acetate of a flocculent gray-brown precipitate, from which may be separated a yellow filtrate; and the small proportion of color insoluble in amyl alcohol. Imitation vanilla extract, prepared ordinarily from artificial vanillin or coumarin, may contain exactly the quantity of vanillin found in genuine extracts, but is readily distinguished by the absence of resins, low lead number, the scanty dark-brown precipitate and brownish filtrate with lead acetate, and the high percentages of color in the lead filtrate and insoluble in amyl alcohol. The presence of coumarin and of vanillin over 0.35 per cent would also be suspicious.

The ash values are useful, on the one hand, because the common ingredients of imitation extracts, vanillin, coumarin, sugar, glycerol, and alcohol, are practically free from ash and, on the other hand, because the use of alkali would not only increase the amount of ash but also change the values for solubility and alkalinity. A marked degree of acidity, due chiefly to organic acids dissolved from the beans and in lesser degree to natural vanillin, is characteristic of genuine vanilla extract, whereas low acidity is indicative of adulteration.

The detection of more skillfully prepared extracts, consisting oftentimes of low-grade Tahiti vanilla beans, reinforced by extract of tonka beans or prune juice, together with the required amount of vanillin to simulate a natural extract, is more difficult.

Probably the best indication of adulteration will be shown by the normal lead number, since this varies less in genuine vanilla than some of the other constants. Since the normal lead number of tonka extract is so low (see Table 78, below), this, taken in connection with the amount of coumarin present, is of value in showing the presence of this adulterant.

TABLE 78.—ANALYSES OF ADULTERATED VANILLA EXTRACTS[1]

Sample	Vanillin, per cent	Coumarin, per cent	Normal lead number	Color insoluble in amyl alcohol, per cent
A	None	0.25	0.11	30.8
B	0.16	0.016	0.50	47.0
C	0.17	None	0.42	52.6
D	0.14	0.04	0.19	90.9
E	0.16	None	0.39	76.9

[1] The samples referred to in the above table were prepared as follows:

A. Extract by U. S. P. method of Angostura tonka beans.

B. Extract adulterated with tonka bean extract and caramel.

C. Vanilla extract (Mexican and Tahiti beans) adulterated with extract of dried prunes and synthetic vanillin, and colored with caramel.

D. A wholly factitious product, containing 25 per cent tonka bean extract (10 per cent tonka beans, 20 per cent sugar, 70 per cent alcohol) and 75 per cent of extract of dried prunes, to which was added synthetic vanillin and caramel.

E. Contained about 40 per cent Tahiti bean extract (of rather inferior grade) adulterated with synthetic vanillin, and colored with caramel.

The color of true vanilla seems to be the most variable constituent and the most difficult of extraction. At the same time it is possibly the constituent most difficult to imitate, especially in its relations to the other constituents. For detecting caramel,

the color most commonly added, the determination of the percentage of color insoluble in amyl alcohol is unquestionably the best test.

Of some help for comparison should be the figures given in the table on page 466 which gives the results obtained by collaborators of the Association of Official Agricultural Chemists on various adulterated extracts.[1]

Lemon Extract

Next in importance to vanilla as regards the extent to which it is used, is extract of lemon. This is defined in the Federal standards[2] as "the flavoring extract prepared from oil of lemon, or from lemon peel, or both, and contains not less than 5 per cent by volume of oil of lemon." The extract was probably prepared originally by macerating lemon peel in alcohol, and a similar preparation is still listed in the U. S. Pharmacopœia under the name of "Tincture of lemon peel." The best quality of extracts on the market now, however, are practically all made by dissolving the requisite quantity of lemon oil in strong "deodorized" alcohol, the resulting product being usually, though not always, colored yellow by the addition of some coloring material. The actual color of the lemon peel is seldom employed, being fugitive to the light. Of late years "non-alcoholic extracts" have been made by emulsifying lemon oil with a mucilage of gum, like acacia or tragacanth, and glycerol. Some of them are also made by dissolving lemon oil in a vegetable oil, as cottonseed, corn, or even a light mineral oil.

Lemon oil, which is the essential ingredient of the extract, is produced by moderate pressure from the peel or rind of the lemon, the largest quantity coming from Sicily.[3] It is a light-yellow liquid, having the characteristic odor of lemons, and is composed chiefly of a terpene, *d-limonene*, and the aldehyde *citral*. The former occurs in greatest amount, comprising approximately 90 per cent of the oil, but the odor and value of the oil are due mainly to its citral content. Other substances present in small amounts, mainly esters and traces of other aldehydes,

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **152**, p. 129.

[2] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

[3] Chace: *Ind. Eng. Chem.*, **1909**, 18.

modify the flavor, however, so that even pure citral is by no means a satisfactory substitute for the genuine oil.

Average values for Sicilian lemon oil are: Sp. gr. (15.5°) = 0.852; n_D^{20} = 1.4748; opt. rot. (20°) = +60.12°;[1] citral (Hiltner method) = 4.2 per cent.

Forms of Adulteration.—In the manufacture of a genuine extract of lemon, the alcohol is the expensive part of the preparation, costing approximately four times as much as the oil. The producer of a cheap extract naturally seeks to reduce the cost by decreasing the proportion of alcohol. Since, however, lemon oil is nearly insoluble in dilute alcohol, such extracts must contain only very little oil, or else be made from "terpeneless" oils, as explained below. Former brands on the market have been found to contain so little lemon oil that it could hardly be detected by chemical tests, being often made by shaking lemon oil with weak alcohol and filtering through magnesia to remove the excess of oil, the result being nothing more than an odor of lemon. A 10-cent bottle of such extract, although colored a beautiful lemon yellow, contains materials costing but a fraction of a cent and is nearly worthless as a flavor.

Since the limonene is the least valuable portion of the oil from the standpoint of flavor and, moreover, is the part of the oil that requires strong alcohol for solution, lemon oils are frequently treated so as to remove the greater part or nearly all of the limonene. This may be done by fractional distillation or by washing the oil with dilute alcohol, by which means the citral is gradually washed out. Such oils are sold as "terpeneless," "soluble," "washed," or "concentrated" oils, and their great advantage is that they can be dissolved in weak alcohol. As intimated above, these oils are inferior in quality as compared with pure oil of lemon, and their use constitutes an adulteration unless the extract is sold under some distinctive name, as "Terpeneless Extract of Lemon" to denote that it is not a genuine extract. It is required in the Federal standards that these terpeneless extracts should contain at least 0.2 per cent of citral, this being the amount equivalent to the presence of 5 per cent of lemon oil, containing 4 per cent of citral.

Minute quantities of highly odorous oils, as oil of lemon grass or oil of citronella, are sometimes used in cheap extracts, but these

[1] Angular degrees.

are readily distinguished by their harsh, verbenalike odor as compared with the pleasant fragrance of the genuine lemon.

Since lemons have naturally a yellow color, the popular conception of lemon extract is that it should have a similar color, so that practically all the brands on the market are colored varying shades of yellow with such colors as turmeric or coaltar dyes. Some of the extracts containing practically no oil are the most highly colored. Since this is an imitation of a product having a natural color of its own, the use of any color except the natural color of the lemon peel, unless its presence is disclosed on the label, constitutes an adulteration or misbranding.

ANALYTICAL METHODS

Preliminary Test.—Place a small quantity of the extract in a test tube and add eight to ten times its volume of water. If an appreciable amount of lemon oil is present a distinct cloud or turbidity will be produced, on account of the insolubility of the oil in water.

Note.—This simple test, which can be readily applied in the household, is in some cases all that is required to show the character of an extract. When no precipitate at all, or at most only a faint cloud, is produced, there can be only a trace of lemon oil present. The formation of a cloud is of course not conclusive evidence that the sample contains genuine lemon oil, but in the absence of one, lemon oil is certainly not present.

Determination of Lemon Oil.[1] *a. By Polarization.*—Polarize the undiluted extract in a 200-mm. tube at 20°C. If the instrument is graduated for the Ventzke sugar scale (see page 287), divide the reading by 3.2; if the angular degree scale is used, divide the reading, expressed in minutes, by 68. In either case, if no other optically active substance is present, the quotient is the percentage of lemon oil by volume.

Note.—Lemon extracts occasionally contain a small amount of cane sugar, it being used to facilitate solution of the oil. In case cane sugar is present, as may readily be found by evaporating a portion of the extract, weigh out 10 grams of the sample, evaporate to dryness on the water bath, wash by decantation with three 5-cc. portions of ether, dry, and weigh. Deduct

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[1] MITCHELL: *J. Am. Chem. Soc.*, **1899**, 1132; U. S. Dept. Agr., *Bur. Chem. Bull.* **107** (Rev.), p. 160.

from the polarization 0.38° for each 0.1 per cent of cane sugar found $\left(0.1 \times \dfrac{100}{26.048} = 0.38\right)$.

The percentage of lemon oil in certain non-alcoholic flavors, where it is in admixture with corn, cottonseed, peanut, or mineral oil, may be determined equally well by the polariscope. Polarize the sample at 20°C. in a 200-mm. tube, making the usual five readings. From the average of these readings, in degrees Ventzke, subtract, for corn oil $+0.6°$, for cottonseed oil $-0.3°$, for peanut oil $+0.2°$, and for mineral oil $+5.5°$, as a correction for the rotatory effect of the menstruum. Divide the corrected reading by the following factors to obtain the percentage by volume of lemon oil: 3.4 for corn oil, 3.7 for cottonseed oil, 3.6 for peanut oil, and 3.5 for mineral oil.

b. By Precipitation.—Measure with a pipette 20 cc. of the extract into a Babcock milk bottle (page 129), add 1 cc. of dilute $(1 + 1)$ hydrochloric acid and 25 cc. of water previously warmed to 60°C. Mix the contents of the bottle and stand it in warm water for 5 minutes. Then centrifuge for 5 minutes. Add enough warm water to bring the oil into the graduated neck of the bottle and centrifuge again for 2 minutes. Stand the bottle in water at 60° for 5 minutes and read off the per cent of oil by volume. If the oil found is more than 2 per cent, add to the amount 0.4 per cent to correct for the oil that does not separate. If the amount is between 1 per cent and 2 per cent, the correction is 0.3 per cent.

Notes.—Since, as stated on page 130, the volume of the graduated portion of the neck of the bottle, from 0 to 10, is 2 cc., if 20 cc. of the extract is taken, the readings will be directly in per cent by volume.

Non-alcoholic lemon flavors, in the form of emulsion, may have the content of oil determined by the same method, if 10 cc. of the emulsion is measured in a graduated cylinder, transferred with small portions of alcohol, using a glass rod, to a 50-cc. graduated flask, shaken thoroughly, allowed to stand for $\frac{1}{2}$ hour, filtered, and the oil determined in a 20-cc. aliquot of the filtrate as detailed above.

Or the oil in such emulsions may be determined by steam distillation.[1] Measure 10 cc. of the emulsion into a graduated

[1] BOYLES: *Ind. Eng. Chem.*, **1918**, 537.

cylinder and transfer it by means of about 35 cc. of water to a side-neck distilling flask and distill with steam into a 100-cc. cassia flask.[1] The method has been found to give consistently a 95 per cent recovery.

In the absence of a polariscope, the lemon oil in vegetable- and mineral-oil flavors may be determined by a somewhat similar distillation process. Since, however, the quantitative recovery of the essential oil is a little more difficult in this case, the details of the apparatus and method, described in the original article,[2] would best be followed.

Alcohol.—In the absence of lemon oil, as indicated by the preliminary test, the alcohol may be determined approximately directly from the specific gravity of the extract, as under Whisky, page 563. If an appreciable amount of oil is present, however, it is better to distill, and in this case the oil must be removed, since otherwise it would pass in part into the distillate. To do this, measure 20 cc. of the extract into a 100-cc. volumetric flask, add water until the mixture rises into the neck of the flask, allow it to stand until the oil separates in a clear layer at the top, then make up to the mark, using the lower meniscus of the oil. Pour the mixture into a dry Erlenmeyer flask containing 5 grams of light magnesium carbonate. Shake thoroughly and filter through a dry filter. Measure out 50 cc. of the clear filtrate, add about 15 cc. of water, and distill 50 cc., following the general procedure as given on page 486. From the specific gravity of the distillate determine the per cent of alcohol by volume and multiply by 5 to obtain the percentage in the original extract.

Notes.—The magnesium carbonate serves to absorb the oil precipitated by the dilution and prevent its passing through the filter.

The distillation of the alcohol after previous extraction of the volatile oil by petroleum ether, as described under Ginger Extract, page 477, can also be used, but for the relatively insoluble lemon oil, the removal by magnesium carbonate is simpler and satisfactory.

If the extract is known to contain only oil, alcohol, and water, the percentage of alcohol can be obtained by a simple calculation

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[1] A volumetric flask with a long neck, graduated 10 cc. in $\frac{1}{10}$-cc. divisions.

[2] *J. Assoc. Off. Agr. Chem.*, **1928**, 45; *Assoc. Off. Agr. Chem.*, "Official Methods," **1935**, p. 317.

with practically the same accuracy as by distilling.[1] Let S represent the specific gravity of the extract (20°C./4°C.), p the percentage of oil, and take 0.86 as the specific gravity of the lemon oil. Then $100 - p$ will be the percentage of the water-alcohol solution, the specific gravity of which, P, is calculated as follows:

$$S = \frac{0.86p + P(100 - p)}{100},$$

whence

$$P = \frac{100S - 0.86p}{100 - p}.$$

The value from the table on page 489, corresponding to P, is the percentage of alcohol in the water-alcohol solution. To find the percentage of alcohol in the extract, multiply this value by $\left(1 - \dfrac{p}{100}\right)$.

Citral.[2] *Reagents. a. Metaphenylenediamine Hydrochloride Solution.*—Dissolve 1 gram of metaphenylenediamine hydrochloride in about 45 cc. of 85 per cent alcohol and 1 gram of crystallized oxalic acid in a similar quantity of alcohol of the same strength and pour the two solutions into a 100-cc. graduated flask. Add 2 or 3 grams of fullers' earth, dilute to the mark with 85 per cent alcohol, mix, and filter through a double-folded filter.

b. Alcohol.—The best quality of cologne spirits will be found suitable for use without further treatment. If, however, the alcohol available is at all colored, or if the metaphenylenediamine solution prepared as above shows a distinct yellow color (due to aldehydes) which is not removed by the fullers' earth, the alcohol used should be treated to remove most of the aldehyde. This can be done readily as described on page 181.

c. Standard Citral Solution.—Dissolve 1 gram of citral in alcohol, 90 to 95 per cent by volume, dilute to 100 cc., and mix. Dilute 5 cc. of this solution to 50 cc. with alcohol, 90 to 95 per cent by volume, and mix. 1 cc. of the second solution = 1 mg. of citral.

[1] HORTVET and WEST: *Ind. Eng. Chem.*, **1909**, 84.
[2] HILTNER: U. S. Dept. Agr., *Bur. Chem. Bull.* **132**, p. 102; PARKER and HILTNER: *Ind. Eng. Chem.*, **1918**, 608.

Method.—All the operations are to be carried on at room temperature. Weigh into a 50-cc. graduated flask 25 grams of the extract and make up to the mark with 95 per cent alcohol. Mix thoroughly, transfer 2 cc. with a pipette to another 50-cc. flask, add 10 cc. of metaphenylenediamine hydrochloride reagent and make up to the mark with alcohol. Compare the color in a Duboscq, or other suitable colorimeter, with that given by 2 cc. of the standard citral solution (1 cc. = 1 mg. of citral) which has been treated with 10 cc. of the reagent and made up to 50 cc. with 95 per cent alcohol in the same way. From this first determination calculate the amount of the standard citral solution that should be used in order that the color may equal approximately that of 2 cc. of the diluted sample and repeat the determination accordingly.

Notes.—The citral used in preparing the standard solution should be redistilled in order to ensure its purity (boiling point is 228°C.) and kept cold and in the dark (best in a refrigerator). Under these conditions it should keep for 6 months with but little change. The dilute standard should be used the same day that it is prepared, since it loses strength.

If the extract contains a considerable amount of cane sugar, the results obtained by the Hiltner method will usually be from 10 to 30 per cent too high, and in this case it will be better to determine the citral by the fuchsin-sulphite method, described on page 566, but it should be remembered that the result will represent the total aldehydes present rather than the citral. Fuchsinsulphurous acid being a general reagent for aldehyde, the small amount of citronellal in the lemon oil is included with the citral.

Color.—Evaporate a portion of the extract to dryness on the water bath to expel alcohol and essential oil, take up the residue in water, and test for coal-tar colors by the double dyeing method described on page 70. In dyeing the color on wool, care should be taken to have the bath only very faintly acid, since Naphthol Yellow S, a color that may be present, being a permitted dye, is decolorized by slight excess of acid. One small drop of acetic or dilute hydrochloric acid will be sufficient. If a coal-tar dye is not found, test for turmeric or lemon-peel color.

Turmeric.—Add to 25 or 50 cc. of the sample 3 drops of saturated boric acid solution, 1 small drop of dilute (1:10) hydrochloric acid, and a piece of filter paper so arranged that it is

only half immersed in the liquid. Evaporate to dryness on the water bath. In the presence of turmeric the paper will be colored pink and the test may be confirmed as described on page 110. Excess of hydrochloric acid should be avoided as in testing for boric acid.

Lemon-peel Color.[1]—Dilute a few cubic centimeters of the extract until the color has nearly disappeared and divide the solution between two test tubes. To one add 3 to 4 volumes of hydrochloric acid and to the other a few drops of strong ammonia. In the presence of the natural color of the lemon peel a distinct yellow color should result in each case.

INTERPRETATION OF RESULTS

The results will usually require but little study in order to distinguish genuine from adulterated extracts. A pure extract

TABLE 79.—ANALYSES OF LEMON EXTRACTS

Statement on label	Alcohol, per cent.	Solids, per cent.	Lemon oil, per cent.	Citral, per cent.	Color	Conclusions of analyst
"Extract of Lemon—Artificially Colored."	86.94	0.2	6.1	0.27	Turmeric.	Genuine lemon extract,
"Pure Concentrated Flavor-Lemon."	47.2	0.07	0.9	0.04	Naphthol Yellow S.	Dilute lemon extract colored.
"Confectioners' Concentration C.X.C. Lemon— Soluble,....Six times the standard strength."	60.64	5.65	1.6	0.56	....	Misbranded as to strength; not concentrated.
"Special Lemon Flavor (Strengthened with Citral)."	45.5	0.10	0.0	0.24	Coal-tar dye.	Imitation lemon flavor prepared from citral, alcohol and color.
"Concentrated Extract of Terpeneless Lemon."	57.0	....	0.4	0.10	Coal-tar dye.	Dilute terpeneless extract.
"Pure Food Extract Lemon (Soluble)—representing a strength of 5 per cent. as required by pure food laws."	29.3	0.04	0.0	0.05	Naphthol Yellow S.	Highly dilute terpeneless extract.
"Double Extract Lemon.".....	7.6	0.8	0.0	0.02	Tartrazine.	Imitation extract containing no true lemon.
"Perfecto Terpeneless Lemon Flavor—Absolutely Pure."	35.2	....	0.0	0.035	Lemon peel.	Less than one-fifth of standard strength.

[1] ALBRECH: U. S. Dept. Agr., *Bur. Chem. Bull.* **137**, p. 71.

will contain at least 5 per cent of lemon oil and ordinarily 80 per cent or more of alcohol. The amount of citral should be between 0.20 and 0.30 per cent. Factitious or imitation extracts usually contain much less alcohol, 20 to 50 per cent, and may show in oil from nearly 5 per cent to none at all. The citral content may be very low (0.02 to 0.05 per cent) in the weak extract, or distinctly higher than normal in extracts that have been reinforced with citral.

Table 79 illustrates typical analyses, taken mainly from the *Notices of Judgment* issued under the Federal Food and Drug Act.

EXTRACT OF GINGER

Ginger extract is defined[1] as "the flavoring extract prepared from ginger and contains in each one hundred (100) cubic centimeters, the alcohol-soluble matters from not less than twenty (20) grams of ginger." The product is practically the same as the "tincture of ginger" of the U. S. Pharmacopœia, the only apparent difference being in the fact that the U. S. P. preparation is required to be made with 95 per cent alcohol, while the definition of the "extract" does not specify the alcoholic strength. It can be readily shown, however, that unless strong alcohol of at least 70 per cent be used, the "alcohol-soluble matters" of the ginger will not be entirely extracted.

The process of the Pharmacopœia for 200 grams of ginger is as follows:

Moisten the ginger with 60 cc. of alcohol, transfer to a percolator and allow it to stand for 6 hours; pack firmly and pour on enough alcohol to saturate the powder. When the liquid begins to drop from the percolator, close the lower orifice, and macerate, covered closely, for 24 hours. Then allow the percolation to proceed slowly, pouring on sufficient alcohol to obtain 1 liter of the tincture.

By this extraction of the powdered ginger with strong alcohol there is obtained a dark-colored aromatic liquid with the characteristic flavor of ginger and containing essentially all of the pungent principles of the root. The extract may also be prepared more simply by dissolving the "oleoresin" of ginger in strong alcohol.

[1] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

This oleoresin, of which ginger contains approximately 5 per cent, is practically the resin, which is of a phenolic nature, dissolved in the essential oil. The pungency of ginger is supposed to be due to the resin whereas the aroma is caused by the essential oil. It may be extracted from the ginger by means of acetone, petroleum ether, or other suitable solvent, which can be distilled off at low temperature, leaving the oleoresin as a viscous liquid. The extract prepared from the oleoresin, if 95 per cent alcohol is used, is almost identical with the U. S. P. tincture.

The exact composition of the extract is dependent to a certain extent upon the kind of ginger employed, Jamaica ginger, which is considered most desirable, not containing on the average so large a proportion of alcohol-soluble matter as some other varieties. As will be shown later, the composition varies considerably with the strength of alcohol employed for extraction, especially if concentrations below 70 per cent by volume are employed.

Forms of Adulteration.—The most common form of adulteration, as in the case of lemon extract, results primarily from economizing in the cost of manufacture by the employment of weak alcohol. In this respect ginger extract is unique, since weak alcohol extracts considerably more than does strong alcohol, the water-soluble material of the root being greatly in excess of the alcohol-soluble. The value of the extract, however, is not to be measured by the total material taken from the ginger by the solvent, but rather by the alcohol-soluble portion, since this will contain the valuable oil and oleoresin, which are insoluble in water. The quantity of dissolved matter present is moreover no criterion of the quality of the extract for the reason that sugar, molasses, glycerol, or similar substances are added to change the flavor of the extract or to give it more "body."

Weak extracts may be helped out in appearance by the use of caramel or other colors and often owe their pungency to the addition of capsicum or extract of cayenne. In some cases ginger may be almost entirely absent, a trace of capsicum in the dilute extract simulating the true ginger.

ANALYTICAL METHODS

Specific Gravity.—Determine at $\frac{20°C.}{20°}$ as described under General Methods, page 4.

Alcohol.—The ordinary methods for determining alcohol directly (see page 486) are not suitable on account of the volatile essential oil. The method used in the case of lemon extract (page 471) can be employed, but if exact results are desired the following method[1] is better:

Dilute 25 cc. of the extract to about ·100 cc., place in a separatory funnel, and add finely powdered salt until saturated. Shake thoroughly for 5 minutes with 50 cc. of light petroleum ether (boiling below 60°C.), let stand for ½ hour, and draw off the lower layer. Wash the petroleum ether twice successively with 25 cc. of saturated salt solution and add to the first aqueous solution, giving a total volume of about 150 cc. Distill 100 cc. and determine the alcohol in the distillate as on page 486. Multiply by 4 to obtain the alcohol in the original sample.

Notes.—The method is a more general one than that described under lemon extracts, being applicable to alcoholic solutions of essential oils which are not precipitated quantitatively by adding water, and hence cannot be removed by magnesium carbonate. It can also be employed for the determination of alcohol in liquids containing such volatile compounds as chloroform, ether, and compound esters, by making two extractions with petroleum ether.

The volatile ginger oil, which would otherwise pass into the distillate with the alcohol, is readily extracted by the petroleum ether especially when "salted out" by the addition of the sodium chloride, its solubility being thereby considerably decreased. The method is a familiar one in many operations of organic chemistry.

Total Solids.—Weigh 10 grams into a flat-bottomed platinum dish and evaporate on the top (not over the live steam) of the water bath. Dry for 2 hours in the drying oven at the temperature of boiling water. Carry out the determination in duplicate.

Solids Soluble in Alcohol.—Add 15 cc. of 95 per cent alcohol to one of the dry residues obtained in the previous determination. Stir thoroughly with a glass rod and allow to stand for 1 hour. Wash into a 50-cc. flask with 95 per cent alcohol and make up to the mark. Filter through a dry fluted filter, evaporate 25 cc., and weigh as in total solids, taking the same precautions to evaporate the alcohol slowly.

[1] THORPE and HOLMES: *J. Chem. Soc.*, **1903**, 314.

Solids Soluble in Water.—Add 15 to 20 cc. of water at room temperature to the other residue obtained in the total solids determination. Stir with a glass rod during 3 hours, taking especial pains to secure intimate mixing with the solvent, which is sometimes difficult on account of the gummy character of the residue. Wash into a 50-cc. flask with water and make up to the mark. Filter through a dry fluted filter, evaporate 25 cc. of the filtrate, and dry to constant weight in a water oven.

Detection of Ginger.[1]—Dilute 10 cc. of the extract to 30 cc., evaporate to 20 cc. to remove the alcohol, transfer to a separatory funnel, and extract with an equal volume of ether. Evaporate the ether spontaneously in a porcelain dish. Add to the residue 10 or 12 drops of concentrated sulphuric acid and about 5 mg. of vanillin crystals, mix thoroughly with a glass rod; allow a few drops of water to flow down the sides of the dish and touch the edge of the acid mixture. A persistent dark-blue color indicates ginger.

Detection of Capsicum.[2]—To 10 cc. of the extract add cautiously dilute (10 per cent) sodium hydroxide until the solution is very slightly alkaline to litmus paper. Evaporate at about 70°C. to approximately one-quarter of the original volume and make slightly acid with dilute sulphuric acid, testing with litmus paper. Transfer to a separatory funnel, rinsing the evaporating dish with water, and extract with an equal volume of ether. Avoid emulsification, shaking the funnel gently for 1 or 2 minutes. Draw off the lower layer and wash the ether extract once with 10 cc. of water. Transfer the washed ether extract to a small evaporating dish, make decidedly alkaline with $0.5N$ alcoholic potash, and evaporate at about 70°C. until the residue is pasty; then add about 20 cc. more of $0.5N$ alcoholic potash and allow to stand on the water bath for $\frac{1}{2}$ hour, or until the gingerol is completely saponified. Dissolve the residue in a little water and transfer with water to a small separatory funnel. The volume should not exceed 50 cc. Extract the alkaline solution with an equal volume of ether and wash the ether until neutral to litmus. Transfer the washed ether to a small evaporating dish

[1] SEEKER: U. S. Dept. Agr., *Bur. Chem. Bull.* **137,** p. 75; MITCHELL: U. S. Dept. Agr., *Bur. Chem. Bull.* **152,** p. 137.

[2] GARNETT and GREEN: *Brit. and Col. Druggist,* **1907**; LAWALL: *Am. J. Pharm.,* **1909,** 218; DOYLE: U. S. Dept. Agr., *Bur. Chem. Bull.* **152,** p. 145.

and allow it to evaporate spontaneously. Finally, test the residue for capsicum by moistening the tip of the finger, rubbing it around on the bottom and sides of the dish and then applying the finger to the end of the tongue. A hot, stinging, or prickly sensation, which persists for several minutes, indicates capsicum.

Notes.—The test depends upon the fact that gingerol, the resin of ginger, to which its pungency is due, is saponified and decomposed by heating with alkali, while *capsaicin*, the active principle of capsicum, remains unaffected. The addition of dilute alkali at first is to prevent the loss of capsaicin by volatilization during the evaporation.

The test is a striking illustration of the great delicacy of some physiological tests as compared with ordinary chemical reactions. There is no color reaction, even, known for capsaicin which will detect the presence of less than 1 part in 1,000, while a distinct "bite" is produced on the tongue by 1 part in 1,000,000.

Detection of Caramel.[1]—The coloring matter of pure ginger extracts is completely soluble in amyl alcohol acidified with phosphoric acid (Marsh reagent), while caramel is insoluble. Hence, carry out the test as described on page 460 and note the resulting colors in the two separated layers of liquid.[2] If the lower or aqueous layer is colorless, caramel is absent. If caramel be present, the lower layer will be colored yellowish brown, the intensity of color being proportional to the amount of caramel present. By comparing the color of the original sample with that of the portion extracted by the Marsh reagent, the approximate amount of added color may be determined.

INTERPRETATION OF RESULTS

Since the standard is based on the presence in the extract of the "alcohol-soluble matters" from a certain proportion of ginger, it is first essential to determine the quantity of such material that would be found in an extract properly prepared. Street and Morison[3] give the following analysis of two samples of U. S. P. ginger tincture prepared in the laboratory.

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[1] HILTNER: U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, p. 91.

[2] It may be necessary if a precipitate forms on diluting the extract on making to volume with 50 per cent alcohol, to compare the color of the aqueous layer with the color of the original extract, rather than with the untreated portion of the diluted extract as directed in Hiltner's test.

[3] U. S. Dept. Agr., *Bur. Chem. Bull.* **137**, p. 76.

Variety	Sp. gr. (15.6°C.)	Alcohol, per cent.	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.
Jamaica.........	0.8198	94.63	1.43	1.42	0.21
African..........	0.8222	93.21	1.81	1.81	0.16

Similar values were found by Lythgoe and Nurenberg[1] for genuine ginger tinctures (Table 80).

TABLE 80.—COMPOSITION OF GENUINE GINGER EXTRACT

Variety	Sp. gr. 20°/4°	Alcohol, per cent.	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.	n_D^{20}
Jamaica.............	0.8184	89.76	1.40	1.33	0.24	1.3662
Jamaica.............	0.8174	89.24	1.38	1.10	0.19	1.3658
Jamaica.............	0.8189	88.84	1.60	1.24	0.22	1.3657
African.............	0.8173	89.36	1.86	1.84	0.08	1.3665
Cochin.............	0.8181	89.24	2.19	1.94	0.22	1.3666
Ginger oleoresin......	0.8144	91.12	1.24	1.16	0.10	1.3668
Average	0.8180	89.29	1.68	1.49	0.19	1.3662

As pointed out on page 476, the determination of total solids alone is not evidence that the extract contains the alcohol-soluble matters of ginger. Alcohol of less strength means a higher percentage of solids in the extract and a greater relative proportion of water-soluble solids. The figures below show the results obtained by decreasing the strength of alcohol used for extraction:

Determination	95 per cent. alcohol	60 per cent. alcohol	20 per cent. alcohol
Total solids......................	1.43	1.91	2.50
Alcohol-soluble solids.............	1.42	1.16	0.30
Water-soluble solids..............	0.21	1.23	2.09

Any concentration of alcohol above 70 per cent, however, gives practically the same results as the 95 per cent alcohol.

Street and Morison suggest that a properly prepared ginger extract should have a specific gravity of about 0.820, and should

[1] *Ind. Eng. Chem.*, **1911**, 910.

contain at least 93 per cent of alcohol by volume and 1 to 2 per cent of solids, practically all of which should be soluble in 95 per cent alcohol, and not over 15 per cent soluble in cold water. In the examination of commercial extracts, however, which are not necessarily prepared by the U. S. P. method, the requirement as to the water-soluble solids should not be interpreted too rigidly. Figure 77 gives the relation between alcohol and solids in ginger extract and shows that the alcohol-soluble solids remain practically the same as the percentage of alcohol decreases, until below 70 per cent, while the water-soluble solids and consequently
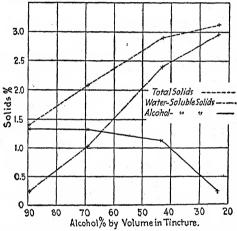
Fig. 77.—Relation of alcohol to solids in tincture of ginger.

the total solids gradually increase.[1] Hence a sample might show a considerable proportion of water-soluble solids and still contain in 100 cc. the specified quantity of "alcohol-soluble matters." The percentage of alcohol-soluble solids is therefore a better index of the quality of the extract and should conform reasonably closely to the values found in the analyses of genuine extracts.

Harrison and Sullivan[2] point out that some of the adulterants of ginger extracts, as molasses, caramel, glycerol, or sugar, are more soluble in alcohol than in ether; hence the percentage of solids soluble in ether conveys more exact information as to the strength of a commercial ginger extract than does the amount of alcohol-soluble solids.

[1] Lythgoe and Nurenberg: *loc. cit.*
[2] *J. Assoc. Off. Agr. Chem.*, **1915**, 506.

To determine the *ether-soluble solids*, 10 cc. is evaporated in a porcelain dish to complete dryness. Absolute ether is then added to the residue, the dish covered with a watch glass and allowed to stand 15 minutes. The ether is then decanted through a dry filter into a tared 100-cc. Erlenmeyer flask, and the ether washing repeated. The undissolved solids remaining in the dish are scraped from the sides with a spatula and rubbed up with successive small portions of ether which are passed through the filter until no more material is dissolved, as shown by the ether coming through colorless. The ether is finally distilled off and the flask dried at 100°C. to constant weight.

TABLE 81.—ANALYSES OF COMMERCIAL GINGER EXTRACTS

Sp. gr. 15.6°	Alcohol, per cent. by volume	Total solids, per cent.	Alcohol-soluble solids, per cent.	Water-soluble solids, per cent.	Remarks
0.8366	96.25	1.85	1.72	0.39	Maximum of 7 standard extracts.
0.8218	90.59	0.94	0.94	0.08	Minimum of 7 standard extracts.
0.8291	93.78	1.38	1.33	0.28	Average of 7 standard extracts.
0.8332	95.07	1.68	1.58	0.47	Maximum of 12 standard tinctures.
0.8224	90.52	1.00	1.00	0.12	Minimum of 12 standard tinctures.
0.8271	93.33	1.36	1.30	0.23	Average of 12 standard tinctures.
0.9184	59.88	2.17	1.12	1.51	Weak alcohol extract.
0.9567	54.28	9.90	1.39	9.12	Contains sugar and water.
0.9948	38.07	10.14	0.86	9.72	Contains molasses and water.
0.9588	37.28	0.49	0.42	0.41	Contains capsicum and water.
0.9557	39.35	1.18	0.54	1.01	Contains oleoresin ginger, oleoresin capsicum, essence oil ginger, caramel and water.
0.9054	54.65	1.42	0.67	1.12	Made from exhausted ginger.
0.9308	46.91	0.73	0.46	0.54 ⎫	Made from oleoresin of ginger and
0.9464	39.52	0.46	0.33	0.29 ⎬	weak alcohol.
0.9864	9.64	0.73	0.16	0.71 ⎫	Made from oleoresin of ginger, cap-
0.9960	2.10	0.43	0.28	0.39 ⎬	sicum and very dilute alcohol.

In a series of extracts prepared from several varieties of ginger, using 95 per cent alcohol, the ratio of alcohol-soluble to total solids varied from 1:1.01 to 1:1.09; and the ether-soluble to total solids from 1:1.2 to 1:1.14. In extracts prepared with 50 per cent alcohol, however, the ratio of alcohol-soluble to total solids was from 1:1.51 to 1:7.00, with a general average of about 1:2.5. In the case of the ether-soluble solids, however, a higher ratio

was found, ranging from 1:3.98 to 1:10.16, being generally above 1:5.

In Table 81 are given some typical analyses of commercial extracts taken from the papers quoted above, including both standard and adulterated samples.

Selected References

GILDEMEISTER and HOFFMANN: "The Volatile Oils," trans. by Kremers, 2d ed., Chemical Catalog (1913–1922).

HORTVET and WEST: Determination of Essential Oils in Flavoring Extracts, *Ind. Eng. Chem.*, **1909**.

PARRY: "The Chemistry of Essential Oils," 4th ed., Scott, London (1922).

WINTON and BERRY: Composition of Authentic Vanilla Extracts, U. S. Dept. Agr., *Bur. Chem. Bull.* **152**.

CHAPTER XI

ALCOHOLIC FOODS

If the term *food* be broadened to include also beverages, then the alcoholic foods rank in analytical importance and interest with the saccharine foods and the fats and oils. It is indeed no severe tax on the imagination to regard the alcoholic beverages as essentially saccharine foods, since alcohol, their chief constituent, is derived by the fermentation of the sugar either naturally present or added. Further, many of them contain notable quantities of sugars still unfermented.

The alcoholic foods group themselves naturally into two great divisions: (*a*) those like wine or beer in which the fermented product is consumed directly; and (*b*) products like whisky in which, for the concentration of the alcohol and modification of the flavor, the fermented product is further subjected to distillation before being consumed.

As with saccharine products and with oils, certain factors common to all will be considered first and then a typical example from each class will be discussed in greater detail.

Alcohol.—By the word *alcohol* without qualification is usually meant ethyl alcohol, a colorless mobile liquid of characteristic odor and burning taste. It is miscible with water in all proportions, the mixing being accompanied by the evolution of heat and a distinct contraction in volume. It has a boiling point of 78.4°C. and a specific gravity of 0.79389 at $\frac{15.56°}{15.56°}$C., as adopted in this country by the Bureau of Standards, based on the work of Mendelejeff.

Detection of Alcohol.—Qualitative tests for ethyl alcohol are not often required in food analysis, since ordinarily its presence is assumed in certain classes of food materials and the quantitative determination can be carried out with ease and rapidity. If, however, such a test is necessary, either or both of those described below will be found satisfactory.

Occasionally the test may be applied directly to the original liquid, but in most cases it will be found better to concentrate it by distillation. This may be done by direct distillation after the addition of salt, by which the alcohol is distilled freer from water, or better by distilling the neutralized liquid through a simple fractionating tower, such as the ordinary Glinsky tube (Fig. 78).

a. Iodoform Test.—To 5 cc. of the first portion of the distillate obtained above add 10 drops of 10 per cent sodium hydroxide, then add from a medicine dropper a solution of iodine in potassium iodide until a very slight permanent yellow color is produced. Let the solution stand several minutes, then shake and note whether any iodoform has separated. If no iodoform separates in the cold, immerse the bulb of a small thermometer in the solution, heat to 60°C. for a minute, and set aside. The presence of alcohol is shown by the gradual formation of a yellowish crystalline precipitate of iodoform, CHI_3, with characteristic odor.
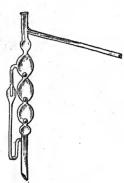
FIG. 78.—Glinsky fractionating tube.

Notes.—Acetone gives the reaction in the cold, ethyl alcohol and several other substances quite readily at 60°; hence a positive test should not be accepted as conclusive evidence of the presence of alcohol, but should be confirmed by the benzoyl chloride test (below) or by the specific gravity or refractive index of the distillate.

An odor of iodoform, unaccompanied by a perceptible precipitate, should not be regarded as a positive test.

b. Ethyl Benzoate Test.—To a small portion of the distillate obtained as described above add a few drops of benzoyl chloride and several cubic centimeters of 10 per cent sodium hydroxide solution. Shake and warm gently. In the presence of ethyl alcohol the ethyl benzoic ester is formed according to the following reaction,

$$C_2H_5OH + C_6H_5COOH \rightarrow C_2H_5O - COC_6H_5 + H_2O,$$

and may be identified by its characteristic odor, and if formed in sufficient quantity by its boiling point (212°C.).

Note.—If a positive result is obtained in the two tests outlined, it may be assumed with reasonable certainty that ethyl alcohol is present, since the only benzoic ester whose odor might be confused with that of the ethyl ester is the methyl ester, and methyl alcohol does not give the iodoform reaction. If further evidence is required it would best be secured from the specific gravity or refractive index of the distillate as described below under the quantitative estimation of alcohol.

Determination of Alcohol.—Measure or weigh (see Notes, page 488) a suitable quantity of the sample into a 500-cc. round-bottomed flask and dilute to 150 cc. With liquids containing more than 25 per cent of alcohol, as distilled liquors or extracts, use 25 cc.; with liquids containing a less percentage of alcohol, as wines, use 100 cc. With some wines or other samples that are distinctly acid, the acidity should be neutralized with dilute sodium hydroxide before distilling, or a pinch (0.1 to 0.2 gram) of precipitated calcium carbonate may be added to the flask. Distill, rather slowly at first, about 95 cc. into a 100-cc. graduated flask with a narrow neck, so arranged that the tip of the condenser is inserted some little distance into the neck of the flask. Fill to the mark (at the proper temperature) with distilled water, mix thoroughly, and take the specific gravity with a pyknometer at $\frac{20°}{20°}$C. or any convenient temperature $\frac{t°}{t°}$C., and convert to $\frac{20°}{4°}$C. by Table 1, page 6. Obtain the corresponding value for alcohol from Table 82, page 489. If desired the refraction of the distillate may be determined by the immersion refractometer and the alcohol figure taken from Table 83, page 503. It is often a good plan to use both methods as a check on each other and to show the possible presence of methyl alcohol (see page 511).

Remember that the value taken from the table is in any case only the percentage of alcohol in the distillate and must still be calculated to the original sample.

Because any error is going to be multiplied, the determination of gravity or refraction must be made with the greatest possible care. Note the precautions given under General Methods, and be especially careful as regards temperature, possible leakage around glass stoppers, deposition of moisture, errors of parallax, and the like.

Notes.—The quantitative separation of absolute alcohol from any food material is practically an impossibility. Hence, the methods commonly used depend upon the examination of a mixture of alcohol and water, separated by distillation from the original material. Methods of examining the distillate other than the determination of its specific gravity comprise the determination of its refractive index, its boiling point, or chemical tests such as oxidation to acetic acid by some suitable oxidizing agent. Of these, the boiling-point method is in general suited only for approximate work, and the last is used only for the estimation of small amounts. For these reasons the specific-gravity and refractometric methods are the only ones considered here in detail.

The method based on the boiling point is worthy of brief mention because it has come into common use in breweries, wineries, and distilleries, especially for control tests. The apparatus used is known as an "ebullioscope" or "ebulliometer," and several forms are approved by the Alcohol Tax Unit of the U. S. Bureau of Internal Revenue. A relatively simple form largely used in the California wine industry is shown in Fig. 79. These instruments have a wide popularity because the procedure is short and simple, and they can be used on a fermented sample without previous distillation. The popular opinion goes even further, holding that it requires no special chemical or technical skill to use one. Such, however,
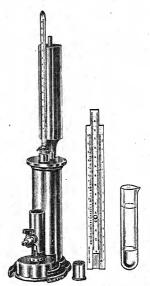
FIG. 79.—Salleron-Dujardin ebullioscope. (*Courtesy of Arthur H. Thomas Co.*)

is far from being the case, the results being decidedly variable with changes in technique, errors in the placing and calibration of the thermometer, and with concentration of alcohol and of dissolved solids in the material examined. A comprehensive report on this type of instrument has been made by Joslyn, Marsh, and Fessler,[1] to which reference should be made for details.

[1] *J. Assoc. Off. Agr. Chem.*, **1937**, 116; **1938**, 175.

The addition of calcium carbonate or of sodium hydroxide in distilling is for the purpose of neutralizing any volatile acid, as acetic, which might be present as the result of fermentation and would otherwise pass into the distillate with the alcohol and water. In many cases the neutralizing will not be necessary.

If the sample contains volatile oils, some of these will of course pass into the distillate, and either the method described on page 471 or that given on page 477, depending on the solubility of the oil in dilute alcohol, should be used instead of simple distillation.

The result is expressed ordinarily in per cent of absolute alcohol by weight or by volume. The former is perhaps more in keeping with the general method of expressing analytical results, but the statement as per cent by volume is required under many of the liquor laws, and under the Federal Pure Food Law, hence is probably more commonly used. Still another method of stating the alcohol content, and that employed in the Bureau of Internal Revenue, is as the percentage of *proof spirit,* by which is meant a liquor containing 50 per cent by volume of alcohol. Thus, a whisky of "96 proof" would contain 48 per cent of alcohol by volume. *British proof spirit* is somewhat different, containing 57.07 per cent of alcohol by volume at 15.6°C. The percentage of alcohol by weight in the distillate can of course be taken directly from the appropriate table. In order to calculate it to the original sample, the weight or the volume and the specific gravity of the latter must be also known.

As a result of the use of different alcohol tables, based on varying data, some confusion has resulted, since the results obtained may be slightly different. That given here, published by the Bureau of Standards, is one of the best. An alcohol table giving percentages of alcohol by volume at apparent specific gravities for a convenient range of temperatures from $\frac{20°}{20°}$ to $\frac{35°}{35°}$ will be found in the 1935 "Official Methods" of the Association of Official Agricultural Chemists. Since in the construction of the table on page 489 all weighings have been reduced to *vacuo,* the weights of distillate and of water contained in the pyknometer should for exact work be corrected for the buoyant effect of the air. This may be done in each case by adding 0.00106 gram for each gram of water or of distillate that the pyknometer apparently contains. Since, however, this correction is applied to both the

TABLE 82.—ALCOHOL TABLE

(Calculated by the U. S. Bureau of Standards from its experimental results[1])

For calculating the percentages of alcohol in mixtures of ethyl alcohol and water from their specific gravities

Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.99823	0.00	0.00	0.00	0.99492	2.25	1.79	1.78	0.99174	4.50	3.58	3.55
0.99815	0.05	0.04	0.04	0.99485	2.30	1.82	1.81	0.99168	4.55	3.62	3.59
0.99808	0.10	0.08	0.08	0.99477	2.35	1.86	1.85	0.99161	4.60	3.66	3.63
0.99800	0.15	0.12	0.12	0.99470	2.40	1.90	1.89	0.99154	4.65	3.70	3.67
0.99793	0.20	0.16	0.16	0.99463	2.45	1.94	1.93	0.99147	4.70	3.74	3.71
0.99785	0.25	0.20	0.20	0.99456	2.50	1.98	1.97	0.99140	4.75	3.78	3.75
0.99778	0.30	0.24	0.24	0.99449	2.55	2.02	2.01	0.99133	4.80	3.82	3.79
0.99770	0.35	0.28	0.28	0.99442	2.60	2.06	2.05	0.99127	4.85	3.86	3.83
0.99763	0.40	0.32	0.32	0.99434	2.65	2.10	2.09	0.99120	4.90	3.90	3.87
0.99755	0.45	0.36	0.36	0.99427	2.70	2.14	2.13	0.99113	4.95	3.94	3.91
0.99748	0.50	0.40	0.40	0.99420	2.75	2.18	2.17	0.99106	5.00	3.98	3.95
0.99741	0.55	0.44	0.44	0.99413	2.80	2.22	2.21	0.99100	5.05	4.02	3.99
0.99734	0.60	0.47	0.47	0.99405	2.85	2.26	2.25	0.99093	5.10	4.06	4.03
0.99726	0.65	0.51	0.51	0.99398	2.90	2.30	2.29	0.99087	5.15	4.10	4.07
0.99719	0.70	0.55	0.55	0.99391	2.95	2.34	2.33	0.99080	5.20	4.14	4.10
0.99711	0.75	0.59	0.59	0.99384	3.00	2.38	2.37	0.99073	5.25	4.18	4.14
0.99704	0.80	0.63	0.63	0.99377	3.05	2.42	2.41	0.99066	5.30	4.22	4.18
0.99697	0.85	0.67	0.67	0.99370	3.10	2.46	2.45	0.99060	5.35	4.26	4.22
0.99690	0.90	0.71	0.71	0.99362	3.15	2.50	2.49	0.99053	5.40	4.30	4.26
0.99682	0.95	0.75	0.75	0.99355	3.20	2.54	2.53	0.99047	5.45	4.34	4.30
0.99675	1.00	0.79	0.79	0.99348	3.25	2.58	2.57	0.99040	5.50	4.38	4.34
0.99667	1.05	0.83	0.83	0.99341	3.30	2.62	2.60	0.99033	5.55	4.42	4.38
0.99660	1.10	0.87	0.87	0.99334	3.35	2.66	2.64	0.99026	5.60	4.46	4.42
0.99652	1.15	0.91	0.91	0.99327	3.40	2.70	2.68	0.99020	5.65	4.50	4.46
0.99645	1.20	0.95	0.95	0.99320	3.45	2.74	2.72	0.99013	5.70	4.54	4.50
0.99638	1.25	0.99	0.99	0.99313	3.50	2.78	2.76	0.99006	5.75	4.58	4.54
0.99631	1.30	1.03	1.03	0.99306	3.55	2.82	2.80	0.98999	5.80	4.62	4.58
0.99623	1.35	1.07	1.07	0.99299	3.60	2.86	2.84	0.98993	5.85	4.66	4.62
0.99616	1.40	1.11	1.11	0.99292	3.65	2.90	2.88	0.98986	5.90	4.70	4.66
0.99608	1.45	1.15	1.15	0.99285	3.70	2.94	2.92	0.98980	5.95	4.74	4.70
0.99601	1.50	1.19	1.19	0.99278	3.75	2.98	2.96	0.98973	6.00	4.78	4.74
0.99594	1.55	1.23	1.23	0.99271	3.80	3.02	3.00	0.98967	6.05	4.82	4.78
0.99587	1.60	1.27	1.26	0.99264	3.85	3.06	3.04	0.98960	6.10	4.87	4.82
0.99579	1.65	1.31	1.30	0.99257	3.90	3.10	3.08	0.98954	6.15	4.91	4.86
0.99572	1.70	1.35	1.34	0.99250	3.95	3.14	3.12	0.98947	6.20	4.95	4.89
0.99564	1.75	1.39	1.38	0.99243	4.00	3.18	3.16	0.98941	6.25	4.99	4.93
0.99557	1.80	1.43	1.42	0.99236	4.05	3.22	3.20	0.98934	6.30	5.03	4.97
0.99550	1.85	1.47	1.46	0.99229	4.10	3.26	3.24	0.98928	6.35	5.07	5.01
0.99543	1.90	1.51	1.50	0.99222	4.15	3.30	3.28	0.98921	6.40	5.11	5.05
0.99535	1.95	1.55	1.54	0.99215	4.20	3.34	3.32	0.98915	6.45	5.15	5.09
0.99528	2.00	1.59	1.58	0.99208	4.25	3.38	3.36	0.98908	6.50	5.19	5.13
0.99520	2.05	1.63	1.62	0.99201	4.30	3.42	3.39	0.98902	6.55	5.23	5.17
0.99513	2.10	1.67	1.66	0.99195	4.35	3.46	3.43	0.98895	6.60	5.27	5.21
0.99506	2.15	1.71	1.70	0.99188	4.40	3.50	3.47	0.98889	6.65	5.31	5.25
0.99499	2.20	1.75	1.74	0.99181	4.45	3.54	3.51	0.98882	6.70	5.35	5.29

[1] *U. S. Bur. Standards Bull.* **9**, No. 3.

Table 82.—Alcohol Table.—(*Continued*)

Specific gravity 20°C./4°	Alcohol Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Alcohol Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Alcohol Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.
0.98876	6.75	5.39	5.33	0.98566	9.25	7.41	7.30	0.98267	11.75	9.44	9.28
0.98870	6.80	5.43	5.37	0.98560	9.30	7.45	7.34	0.98261	11.80	9.48	9.31
0.98864	6.85	5.47	5.41	0.98554	9.35	7.49	7.38	0.98255	11.85	9.52	9.35
0.98857	6.90	5.51	5.45	0.98549	9.40	7.53	7.42	0.98250	11.90	9.56	9.39
0.98851	6.95	5.55	5.49	0.98543	9.45	7.57	7.46	0.98244	11.95	9.60	9.43
0.98845	7.00	5.59	5.53	0.98537	9.50	7.61	7.50	0.98238	12.00	9.64	9.47
0.98839	7.05	5.63	5.57	0.98531	9.55	7.65	7.54	0.98232	12.05	9.68	9.51
0.98832	7.10	5.67	5.60	0.98524	9.60	7.69	7.58	0.98226	12.10	9.72	9.55
0.98826	7.15	5.71	5.64	0.98518	9.65	7.73	7.62	0.98220	12.15	9.76	9.59
0.98820	7.20	5.75	5.68	0.98512	9.70	7.77	7.66	0.98214	12.20	9.80	9.63
0.98813	7.25	5.79	5.72	0.98506	9.75	7.81	7.70	0.98208	12.25	9.84	9.67
0.98806	7.30	5.83	5.76	0.98501	9.80	7.85	7.73	0.98203	12.30	9.89	9.71
0.98800	7.35	5.87	5.80	0.98495	9.85	7.89	7.77	0.98197	12.35	9.93	9.75
0.98794	7.40	5.91	5.84	0.98488	9.90	7.93	7.81	0.98191	12.40	9.97	9.79
0.98788	7.45	5.95	5.88	0.98482	9.95	7.97	7.85	0.98185	12.45	10.01	9.83
0.98781	7.50	5.99	5.92	0.98476	10.00	8.02	7.89	0.98180	12.50	10.05	9.87
0.98775	7.55	6.03	5.96	0.98470	10.05	8.06	7.93	0.98174	12.55	10.09	9.91
0.98769	7.60	6.07	6.00	0.98463	10.10	8.10	7.97	0.98168	12.60	10.13	9.95
0.98763	7.65	6.11	6.04	0.98457	10.15	8.14	8.01	0.98162	12.65	10.17	9.99
0.98756	7.70	6.15	6.08	0.98452	10.20	8.18	8.05	0.98156	12.70	10.21	10.03
0.98750	7.75	6.19	6.12	0.98446	10.25	8.22	8.09	0.98150	12.75	10.25	10.07
0.98744	7.80	6.24	6.16	0.98441	10.30	8.26	8.13	0.98145	12.80	10.29	10.10
0.98738	7.85	6.28	6.20	0.98435	10.35	8.30	8.17	0.98139	12.85	10.33	10.14
0.98731	7.90	6.32	6.24	0.98428	10.40	8.34	8.21	0.98132	12.90	10.38	10.18
0.98725	7.95	6.36	6.28	0.98422	10.45	8.38	8.25	0.98127	12.95	10.42	10.22
0.98718	8.00	6.40	6.32	0.98416	10.50	8.42	8.29	0.98122	13.00	10.46	10.26
0.98712	8.05	6.44	6.36	0.98410	10.55	8.46	8.33	0.98116	13.05	10.50	10.30
0.98706	8.10	6.48	6.39	0.98404	10.60	8.50	8.37	0.98111	13.10	10.54	10.34
0.98700	8.15	6.52	6.43	0.98398	10.65	8.54	8.41	0.98105	13.15	10.58	10.38
0.98694	8.20	6.56	6.47	0.98391	10.70	8.58	8.45	0.98100	13.20	10.62	10.42
0.98688	8.25	6.60	6.51	0.98385	10.75	8.62	8.49	0.98094	13.25	10.66	10.46
0.98682	8.30	6.64	6.55	0.98379	10.80	8.66	8.52	0.98089	13.30	10.70	10.50
0.98676	8.35	6.68	6.59	0.98373	10.85	8.70	8.56	0.98083	13.35	10.74	10.54
0.98670	8.40	6.72	6.63	0.98368	10.90	8.75	8.60	0.98077	13.40	10.78	10.58
0.98664	8.45	6.76	6.67	0.98362	10.95	8.79	8.64	0.98071	13.45	10.82	10.62
0.98658	8.50	6.80	6.71	0.98356	11.00	8.83	8.68	0.98066	13.50	10.86	10.66
0.98652	8.55	6.84	6.75	0.98350	11.05	8.87	8.72	0.98060	13.55	10.90	10.70
0.98646	8.60	6.88	6.79	0.98344	11.10	8.91	8.76	0.98054	13.60	10.95	10.74
0.98640	8.65	6.92	6.83	0.98338	11.15	8.95	8.80	0.98048	13.65	10.99	10.78
0.98633	8.70	6.96	6.97	0.98332	11.20	8.99	8.84	0.98043	13.70	11.03	10.81
0.98627	8.75	7.00	6.91	0.98326	11.25	9.03	8.88	0.98037	13.75	11.07	10.85
0.98620	8.80	7.04	6.95	0.98320	11.30	9.07	8.92	0.98031	13.80	11.11	10.89
0.98614	8.85	7.08	6.99	0.98314	11.35	9.11	8.96	0.98025	13.85	11.15	10.93
0.98608	8.90	7.12	7.03	0.98308	11.40	9.15	9.00	0.98020	13.90	11.19	10.97
0.98602	8.95	7.16	7.07	0.98302	11.45	9.19	9.04	0.98014	13.95	11.23	11.01
0.98596	9.00	7.20	7.10	0.98296	11.50	9.23	9.08	0.98009	14.00	11.28	11.05
0.98590	9.05	7.24	7.14	0.98290	11.55	9.27	9.12	0.98003	14.05	11.32	11.09
0.98584	9.10	7.29	7.18	0.98285	11.60	9.32	9.16	0.97998	14.10	11.36	11.13
0.98578	9.15	7.33	7.22	0.98279	11.65	9.36	9.20	0.97992	14.15	11.40	11.17
0.98572	9.20	7.37	7.26	0.98273	11.70	9.40	9.24	0.97987	14.20	11.44	11.21

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.97980	14.25	11.48	11.25	0.97704	16.75	13.53	13.22	0.97438	19.25	15.59	15.20
0.97975	14.30	11.52	11.29	0.97699	16.80	13.57	13.26	0 97433	19.30	15.64	15.23
0.97969	14.35	11.56	11.33	0.97694	16.85	13.61	13.30	0.97428	19.35	15.68	15.27
0.97964	14.40	11.60	11.37	0.97689	16.90	13.66	13.34	0.97423	19.40	15.72	15.31
0.97958	14.45	11.64	11.41	0.97683	16.95	13.70	13.38	0.97417	19.45	15.76	15.35
0.97953	14.50	11.68	11.44	0.97678	17.00	13.74	13.42	0.97412	19.50	15.80	15.39
0.97947	14.55	11.72	11.48	0.97672	17.05	13.78	13.46	0.97407	19.55	15.84	15.43
0.97942	14.60	11.77	11.52	0.97667	17.10	13.82	13.50	0.97402	19.60	15.88	15.47
0.97936	14.65	11.81	11.56	0.97661	17.15	13.86	13.54	0.97396	19.65	15.92	15.51
0.97930	14.70	11.85	11.60	0.97656	17.20	13.90	13.58	0.97391	19.70	15.97	15.55
0.97924	14.75	11.89	11.64	0.97650	17.25	13.94	13.62	0.97386	19.75	16.01	15.59
0.97919	14.80	11.93	11.68	0.97645	17.30	13.98	13.66	0.97381	19.80	16.05	15.63
0.97913	14.85	11.97	11.72	0.97639	17.35	14.02	13.70	0.97375	19.85	16.09	15.67
0.97908	14.90	12.01	11.76	0.97634	17.40	14.07	13.74	0.97370	19.90	16.13	15.71
0.97902	14.95	12.05	11.80	0.97629	17.45	14.11	13.78	0.97364	19.95	16.17	15.75
0.97897	15.00	12.09	11.84	0.97624	17.50	14.15	13.81	0.97359	20.00	16.21	15.79
0.97891	15.05	12.13	11.88	0.97618	17.55	14.19	13.85	0.97354	20.05	16.25	15.83
0.97885	15.10	12.18	11.92	0.97613	17.60	14.23	13.89	0.97349	20.10	16.30	15.87
0.97879	15.15	12.22	11.96	0.97607	17.65	14.27	13.93	0.97344	20.15	16.34	15.91
0.97874	15.20	12.26	12.00	0.97602	17.70	14.31	13.97	0.97339	20.20	16.38	15.95
0.97868	15.25	12.30	12.04	0.97596	17.75	14.35	14.01	0.97333	20.25	16.42	15.99
0.97863	15.30	12.34	12.08	0.97591	17.80	14.40	14.05	0.97328	20.30	16.46	16.02
0.97857	15.35	12.38	12.12	0.97586	17.85	14.44	14.09	0.97322	20.35	16.50	16.06
0.97852	15.40	12.42	12.16	0.97581	17.90	14.48	14.13	0.97317	20.40	16.55	16.10
0.97846	15.45	12.46	12.20	0.97575	17.95	14.52	14.17	0.97311	20.45	16.59	16.14
0.97841	15.50	12.50	12.23	0.97570	18.00	14.56	14.21	0.97306	20.50	16.63	16.18
0.97835	15.55	12.54	12.27	0.97564	18.05	14.60	14.25	0.97300	20.55	16.67	16.22
0.97830	15.60	12.59	12.31	0.97559	18.10	14.64	14.29	0.97295	20.60	16.71	16.26
0.97824	15.65	12.63	12.35	0.97553	18.15	14.68	14.33	0.97289	20.65	16.75	16.30
0.97819	15.70	12.67	12.39	0.97548	18.20	14.73	14.37	0.97284	20.70	16.80	16.34
0.97813	15.75	12.71	12.43	0.97542	18.25	14.77	14.41	0.97278	20.75	16.84	16.38
0.97808	15.80	12.75	12.47	0.97538	18.30	14.81	14.45	0.97273	20.80	16.88	16.42
0.97802	15.85	12.79	12.51	0.97532	18.35	14.85	14.49	0.97268	20.85	16.92	16.46
0.97797	15.90	12.83	12.55	0.97527	18.40	14.89	14.52	0.97163	20.90	16.96	16.50
0.97791	15.95	12.87	12.59	0.97522	18.45	14.93	14.56	0.97257	20.95	17.00	16.54
0.97786	16.00	12.92	12.63	0.97517	18.50	14.97	14.60	0.97252	21.00	17.04	16.58
0.97780	16.05	12.96	12.67	0.97512	18.55	15.01	14.64	0.97247	21.05	17.08	16.62
0.97775	16.10	13.00	12.71	0.97507	18.60	15.06	14.68	0.97242	21.10	17.12	16.66
0.97769	16.15	13.04	12.75	0.97501	18.65	15.10	14.72	0.97237	21.15	17.17	16.70
0.97764	16.20	13.08	12.79	0.97496	18.70	15.14	14.76	0.97232	21.20	17.21	16.73
0.97758	16.25	13.12	12.83	0.97490	18.75	15.18	14.80	0.97227	21.25	17.25	16.77
0.97753	16.30	13.16	12.87	0.97485	18.80	15.22	14.84	0.97222	21.30	17.29	16.81
0.97747	16.35	13.20	12.91	0.97479	18.85	15.26	14.88	0.97216	21.35	17.33	16.85
0.97742	16.40	13.24	12.95	0.97474	18.90	15.30	14.92	0.97210	21.40	17.38	16.89
0.97737	16.45	13.28	12.99	0.97469	18.95	15.34	14.96	0.97204	21.45	17.42	16.93
0.97732	16.50	13.33	13.02	0.97464	19.00	15.39	15.00	0.97199	21.50	17.46	16.97
0.97726	16.55	13.37	13.06	0.97459	19.05	15.43	15.04	0.97193	21.55	17.50	17.01
0.97721	16.60	13.41	13.10	0.97454	19.10	15.47	15.08	0.97188	21.60	17.54	17.05
0.97715	16.65	13.45	13.14	0.97449	19.15	15.51	15.12	0.97183	21.65	17.58	17.09
0.97710	16.70	13.49	13.18	0.97444	19.20	15.55	15.16	0.97178	21.70	17.63	17.13

TABLE 82.—ALCOHOL TABLE.—(Continued)

Specific gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.	Specific Gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.	Specific Gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.97172	21.75	17.67	17.17	0.96896	24.25	19.75	19.14	0.96612	26.75	21.85	21.12
0.97167	21.80	17.71	17.21	0.96891	24.30	19.80	19.18	0.96606	26.80	21.90	21.16
0.97161	21.85	17.75	17.25	0.96885	24.35	19.84	19.22	0.96600	26.85	21.94	21.20
0.97156	21.90	17.79	17.29	0.96880	24.40	19.88	19.26	0.96595	26.90	21.98	21.23
0.97150	21.95	17.83	17.33	0.96874	24.45	19.92	19.30	0.96589	26.95	22.02	21.27
0.97145	22.00	17.88	17.37	0.96869	24.50	19.96	19.34	0.96583	27.00	22.07	21.31
0.97139	22.05	17.92	17.41	0.96863	24.55	20.00	19.38	0.96577	27.05	22.11	21.35
0.97134	22.10	17.96	17.45	0.96857	24.60	20.05	19.42	0.96571	27.10	22.15	21.39
0.97128	22.15	18.00	17.49	0.96851	24.65	20.09	19.46	0.96565	27.15	22.19	21.43
0.97123	22.20	18.04	17.52	0.96846	24.70	20.13	19.50	0.96559	27.20	22.24	21.47
0.97118	22.25	18.08	17.56	0.96840	24.75	20.17	19.54	0.96553	27.25	22.28	21.51
0.97113	22.30	18.13	17.60	0.96835	24.80	20.22	19.58	0.96548	27.30	22.32	21.55
0.97107	22.35	18.17	17.64	0.96829	24.85	20.26	19.62	0.96542	27.35	22.36	21.59
0.97102	22.40	18.21	17.68	0.96823	24.90	20.30	19.66	0.96536	27.40	22.40	21.63
0.97096	22.45	18.25	17.72	0.96817	24.95	20.34	19.70	0.96530	27.45	22.44	21.67
0.97091	22.50	18.29	17.76	0.96812	25.00	20.38	19.73	0.96525	27.50	22.49	21.71
0.97085	22.55	18.33	17.80	0.96806	25.05	20.42	19.77	0.96519	27.55	22.53	21.75
0.97080	22.60	18.38	17.84	0.96801	25.10	20.47	19.81	0.96513	27.60	22.57	21.79
0.97074	22.65	18.42	17.88	0.96795	25.15	20.51	19.85	0.96507	27.65	22.61	21.83
0.97069	22.70	18.46	17.92	0.96789	25.20	20.55	19.89	0.96501	27.70	22.66	21.87
0.97063	22.75	18.50	17.96	0.96783	25.25	20.59	19.93	0.96495	27.75	22.70	21.91
0.97058	22.80	18.54	18.00	0.96778	25.30	20.64	19.97	0.96489	27.80	22.74	21.94
0.97052	22.85	18.58	18.04	0.96772	25.35	20.68	20.01	0.96483	27.85	22.78	21.98
0.97047	22.90	18.63	18.08	0.96766	25.40	20.72	20.05	0.96477	27.90	22.83	22.02
0.97041	22.95	18.67	18.12	0.96760	25.45	20.76	20.09	0.96471	27.95	22.87	22.06
0.97036	23.00	18.71	18.16	0.96755	25.50	20.80	20.13	0.96465	28.00	22.91	22.10
0.97030	23.05	18.75	18.20	0.96749	25.55	20.84	20.17	0.96459	28.05	22.95	22.14
0.97025	23.10	18.79	18.24	0.96744	25.60	20.89	20.21	0.96454	28.10	23.00	22.18
0.97019	23.15	18.83	18.28	0.96738	25.65	20.93	20.25	0.96448	28.15	23.04	22.22
0.97013	23.20	18.88	18.31	0.96733	25.70	20.97	20.29	0.96442	28.20	23.08	22.26
0.97007	23.25	18.92	18.35	0.96727	25.75	21.01	20.33	0.96436	28.25	23.12	22.30
0.97002	23.30	18.96	18.39	0.96722	25.80	21.06	20.37	0.96430	28.30	23.17	22.34
0.96996	23.35	19.00	18.43	0.96716	25.85	21.10	20.41	0.96424	28.35	23.21	22.38
0.96991	23.40	19.04	18.47	0.96710	25.90	21.14	20.44	0.96418	28.40	23.25	22.42
0.96985	23.45	19.08	18.51	0.96704	25.95	21.18	20.48	0.96412	28.45	23.29	22.46
0.96980	23.50	19.13	18.55	0.96699	26.00	21.22	20.52	0.96406	28.50	23.33	22.50
0.96974	23.55	19.17	18.59	0.96693	26.05	21.26	20.56	0.96400	28.55	23.37	22.54
0.96969	23.60	19.21	18.63	0.96687	26.10	21.31	20.60	0.96393	28.60	23.42	22.57
0.96963	23.65	19.25	18.67	0.96681	26.15	21.35	20.64	0.96387	28.65	23.46	22.61
0.96958	23.70	19.29	18.71	0.96675	26.20	21.39	20.68	0.96381	28.70	23.51	22.65
0.96952	23.75	19.33	18.75	0.96669	26.25	21.43	20.72	0.96375	28.75	23.55	22.69
0.96947	23.80	19.38	18.79	0.96664	26.30	21.48	20.76	0.96369	28.80	23.59	22.73
0.96941	23.85	19.42	18.83	0.96658	26.35	21.52	20.80	0.96363	28.85	23.63	22.77
0.96936	23.90	19.46	18.87	0.96653	26.40	21.56	20.84	0.96357	28.90	23.67	22.81
0.96930	23.95	19.50	18.91	0.96647	26.45	21.60	20.88	0.96351	28.95	23.71	22.85
0.96925	24.00	19.55	18.94	0.96641	26.50	21.64	20.92	0.96346	29.00	23.76	22.89
0.96919	24.05	19.59	18.98	0.96635	26.55	21.68	20.96	0.96340	29.05	23.80	22.93
0.96913	24.10	19.63	19.02	0.96630	26.60	21.73	21.00	0.96334	29.10	23.84	22.97
0.96907	24.15	19.67	19.06	0.96624	26.65	21.77	21.04	0.96328	29.15	23.88	23.01
0.96902	24.20	19.71	19.10	0.96618	26.70	21.81	21.08	0.96322	29.20	23.93	23.05

TABLE 82.—ALCOHOL TABLE.—(Continued)

Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.
0.96316	29.25	23.97	23.09	0.96005	31.75	26.10	25.06	0.95669	34.25	28.26	27.03
0.96310	29.30	24.01	23.13	0.95998	31.80	26.15	25.10	0.95662	34.30	28.30	27.07
0.96304	29.35	24.05	23.17	0.95992	31.85	26.19	25.14	0.95655	34.35	28.34	27.11
0.96297	29.40	24.10	23.21	0.95985	31.90	26.23	25.18	0.95648	34.40	28.39	27.15
0.96291	29.45	24.14	23.25	0.95979	31.95	26.27	25.22	0.95641	34.45	28.43	27.19
0.96285	29.50	24.18	23.29	0.95972	32.00	26.32	25.26	0.95634	34.50	28.48	27.23
0.96279	29.55	24.22	23.33	0.95965	32.05	26.36	25.30	0.95627	34.55	28.52	27.27
0.96273	29.60	24.27	23.36	0.95958	32.10	26.41	25.34	0.95619	34.60	28.56	27.31
0.96267	29.65	24.31	23.40	0.95952	32.15	26.45	25.38	0.95612	34.65	28.60	27.35
0.96261	29.70	24.35	23.44	0.95945	32.20	26.49	25.42	0.95605	34.70	28.65	27.39
0.96255	29.75	24.39	23.48	0.95939	32.25	26.53	25.46	0.95598	34.75	28.69	27.43
0.96248	29.80	24.44	23.52	0.95932	32.30	26.58	25.50	0.95591	34.80	28.74	27.47
0.96242	29.85	24.48	23.56	0.95926	32.35	26.62	25.54	0.95584	34.85	28.78	27.51
0.96236	29.90	24.52	23.60	0.95920	32.40	26.66	25.58	0.95577	34.90	28.82	27.55
0.96230	29.95	24.56	23.64	9.95913	32.45	26.70	25.61	0.95570	34.95	28.86	27.59
0.96224	30.00	24.61	23.68	0.95906	32.50	26.75	25.64	0.95563	35.00	28.91	27.63
0.96218	30.05	24.65	23.72	9.95900	32.55	26.79	25.68	0.95556	35.05	28.95	27.67
0.96211	30.10	24.69	23.76	0.95893	32.60	26.83	25.72	0.95549	35.10	29.00	27.71
0.96205	30.15	24.73	23.80	0.95887	32.65	26.87	25.76	0.95542	35.15	29.04	27.75
0.96199	30.20	24.78	23.84	0.95880	32.70	26.92	25.80	0.95535	35.20	29.08	27.78
0.96193	30.25	24.82	23.88	0.95873	32.75	26.96	25.84	0.95528	35.25	29.12	27.82
0.96187	30.30	24.87	23.92	0.95866	32.80	27.01	25.89	0.95521	35.30	29.17	27.86
0.96181	30.35	24.91	23.96	0.95859	32.85	27.05	25.93	0.95513	35.35	29.21	27.90
0.96175	30.40	24.95	24.00	0.95852	32.90	27.09	25.97	0.95506	35.40	29.26	27.94
0.96169	30.45	24.99	24.04	0.95846	32.95	27.13	26.01	0.95499	35.45	29.30	27.98
0.96163	30.50	25.04	24.08	0.95839	33.00	27.18	26.05	0.95492	35.50	29.34	28.02
0.96157	30.55	25.08	24.12	0.95833	33.05	27.22	26.09	0.95485	35.55	29.38	28.06
0.96150	30.60	25.12	24.15	0.95826	33.10	27.27	26.13	0.95478	35.60	29.43	28.10
0.96144	30.65	25.16	24.19	0.95819	33.15	27.31	26.17	0.95470	35.65	29.47	28.14
0.96138	30.70	25.21	24.23	0.95812	33.20	27.35	26.21	0.95463	35.70	29.52	28.18
0.96132	30.75	25.25	24.27	0.95806	33.25	27.39	26.25	0.95456	35.75	29.56	28.22
0.96125	30.80	25.30	24.31	0.95799	33.30	27.44	26.29	0.95449	35.80	29.61	28.26
0.96119	30.85	25.34	24.35	0.95792	33.35	27.48	26.33	0.95441	35.85	29.65	28.30
0.96112	30.90	25.38	24.39	0.95785	33.40	27.52	26.36	0.95434	35.90	29.69	28.34
0.96106	30.95	25.42	24.43	0.95778	33.45	27.56	26.40	0.95426	35.95	29.73	28.38
0.96100	31.00	25.46	24.47	0.95771	33.50	27.61	26.44	0.95419	36.00	29.78	28.42
0.96094	31.05	25.50	24.51	0.95764	33.55	27.65	26.48	0.95412	36.05	29.82	28.46
0.96088	31.10	25.55	24.55	0.95757	33.60	27.70	26.52	0.95405	36.10	29.87	28.49
0.96082	31.15	25.59	24.59	0.95751	33.65	27.74	26.56	0.95397	36.15	29.91	28.53
0.96075	31.20	25.63	24.63	0.95745	33.70	27.78	26.60	0.95390	36.20	29.95	28.57
0.96069	31.25	25.67	24.67	0.95738	33.75	27.82	26.64	0.95382	36.25	29.99	28.61
0.96062	31.30	25.72	24.71	0.95731	33.80	27.87	26.68	0.95375	36.30	30.04	28.65
0.96056	31.35	25.76	24.75	0.95724	33.85	27.91	26.72	0.95367	36.35	30.09	28.69
0.96049	31.40	25.81	24.79	0.95717	33.90	27.96	26.76	0.95360	36.40	30.13	28.73
0.96043	31.45	25.85	24.83	0.95710	33.95	28.00	26.80	0.95353	36.45	30.17	28.77
0.96036	31.50	25.89	24.86	0.95703	34.00	28.04	26.84	0.95346	36.50	30.22	28.81
0.96030	31.55	25.93	24.90	0.95696	34.05	28.08	26.88	0.95338	36.55	30.26	28.85
0.96024	31.60	25.98	24.94	0.95689	34.10	28.13	26.92	0.95331	36.60	30.31	28.89
0.96018	31.65	26.02	24.98	0.95682	34.15	28.17	26.96	0.95323	36.65	30.35	28.93
0.96011	31.70	26.06	25.02	0.95675	34.20	28.22	26.99	0.95315	36.70	30.39	28.97

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.95308	36.75	30.43	29.01	0.94926	39.25	32.63	30.99	0.94519	41.75	34.86	32.96
0.95301	36.80	30.48	29.05	0.94918	39.30	32.68	31.02	0.94510	41.80	34.91	33.00
0.95294	36.85	30.52	29.09	0.94910	39.35	32.72	31.06	0.94502	41.85	34.95	33.04
0.95287	36.90	30.57	29.13	0.94901	39.40	32.77	31.10	0.94494	41.90	35.00	33.07
0.95279	36.95	30.61	29.17	0.94893	39.45	32.81	31.14	0.94486	41.95	35.04	33.11
0.95272	37.00	30.66	29.21	0.94885	39.50	32.86	31.18	0.94477	42.00	35.09	33.15
0.95264	37.05	30.70	29.25	0.94877	39.55	32.90	31.22	0.94469	42.05	35.13	33.19
0.95257	37.10	30.74	29.29	0.94869	39.60	32.95	31.26	0.94460	42.10	35.18	33.23
0.95249	37.15	30.78	29.33	0.94861	39.65	32.99	31.30	0.94452	42.15	35.22	33.27
0.95242	37.20	30.83	29.36	0.94853	39.70	33.04	31.34	0.94443	42.20	35.27	33.31
0.95234	37.25	30.87	29.40	0.94845	39.75	33.08	31.38	0.94435	42.25	35.31	33.35
0.95227	37.30	30.92	29.44	0.94837	39.80	33.13	31.42	0.94427	42.30	35.36	33.39
0.95219	37.35	30.96	29.48	0.94829	39.85	33.17	31.46	0.94419	42.35	35.40	33.43
0.95211	37.40	31.01	29.52	0.94821	39.90	33.22	31.50	0.94410	42.40	35.45	33.47
0.95203	37.45	31.05	29.56	0.94813	39.95	33.26	31.54	0.94402	42.45	35.49	33.51
0.95196	37.50	31.09	29.60	0.94805	40.00	33.30	31.57	0.94393	42.50	35.54	33.55
0.95188	37.55	31.13	29.64	0.94797	40.05	33.34	31.61	0.94385	42.55	35.58	33.59
0.95181	37.60	31.18	29.68	0.94789	40.10	33.39	31.65	0.94376	42.60	35.63	33.63
0.95173	37.65	31.22	29.72	0.94781	40.15	33.43	31.69	0.94368	42.65	35.67	33.67
0.95166	37.70	31.27	29.76	0.94773	40.20	33.48	31.73	0.94359	42.70	35.72	33.71
0.95158	37.75	31.31	29.80	0.94765	40.25	33.52	31.77	0.94351	42.75	35.76	33.75
0.95151	37.80	31.36	29.84	0.94757	40.30	33.57	31.81	0.94342	42.80	35.81	33.78
0.95143	37.85	31.40	29.88	0.94749	40.35	33.61	31.85	0.94334	42.85	35.85	33.82
0.95135	37.90	31.45	29.92	0.94741	40.40	33.66	31.89	0.94325	42.90	35.90	33.86
0.95127	37.95	31.49	29.96	0.94733	40.45	33.70	31.93	0.94317	42.95	35.94	33.90
0.95120	38.00	31.53	29.99	0.94725	40.50	33.75	31.97	0.94308	43.00	35.99	33.94
0.95112	38.05	31.57	30.03	0.94717	40.55	33.79	32.01	0.94300	43.05	36.03	33.98
0.95104	38.10	31.62	30.07	0.94708	40.60	33.84	32.05	0.94291	43.10	36.08	34.02
0.95096	38.15	31.66	30.11	0.94700	40.65	33.88	32.09	0.94283	43.15	36.12	34.06
0.95089	38.20	31.71	30.15	0.94692	40.70	33.93	32.13	0.94274	43.20	36.17	34.10
0.95081	38.25	31.75	30.19	0.94684	40.75	33.97	32.17	0.94265	43.25	36.21	34.14
0.95074	38.30	31.80	30.23	0.94676	40.80	34.02	32.20	0.94256	43.30	36.26	34.18
0.95066	38.35	31.84	30.27	0.94668	40.85	34.06	32.24	0.94248	43.35	36.30	34.22
0.95058	38.40	31.89	30.31	0.94659	40.90	34.11	32.28	0.94239	43.40	36.35	34.26
0.95050	38.45	31.93	30.35	0.94651	40.95	34.15	32.32	0.94231	43.45	36.39	34.30
0.95043	38.50	31.97	30.39	0.94643	41.00	34.19	32.36	0.94222	43.50	36.44	34.34
0.95035	38.55	32.01	30.43	0.94635	41.05	34.23	32.40	0.94214	43.55	36.48	34.38
0.95027	38.60	32.06	30.47	0.94627	41.10	34.28	32.44	0.94205	43.60	36.53	34.42
0.95019	38.65	32.10	30.51	0.94619	41.15	34.32	32.48	0.94197	43.65	36.57	34.46
0.95011	38.70	32.15	30.55	0.94610	41.20	34.37	32.52	0.94188	43.70	36.62	34.49
0.95003	38.75	32.19	30.59	0.94602	41.25	34.41	32.56	0.94179	43.75	36.66	34.53
0.94996	38.80	32.24	30.63	0.94594	41.30	34.46	32.60	0.94170	43.80	36.71	34.57
0.94988	38.85	32.28	30.67	0.94586	41.35	34.50	32.64	0.94161	43.85	36.75	34.61
0.94980	38.90	32.33	30.71	0.94577	41.40	34.55	32.68	0.94152	43.90	36.80	34.65
0.94972	38.95	32.37	30.75	0.94569	41.45	34.59	32.72	0.94144	43.95	36.84	34.69
0.94964	39.00	32.42	30.79	0.94560	41.50	34.64	32.76	0.94135	44.00	36.89	34.72
0.94956	39.05	32.46	30.83	0.94552	41.55	34.68	32.80	0.94126	44.05	36.94	34.77
0.94949	39.10	32.51	30.87	0.94544	41.60	34.73	32.84	0.94117	44.10	36.99	34.81
0.94941	39.15	32.55	30.91	0.94536	41.65	34.77	32.88	0.94108	44.15	37.03	34.85
0.94934	39.20	32.59	30.95	0.94527	41.70	34.82	32.92	0.94099	44.20	37.08	34.89

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity $\frac{20°C.}{4°}$	Alcohol			Specific gravity $\frac{20°C.}{4°}$	Alcohol			Specific gravity $\frac{20°C.}{4°}$	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.94091	44.25	37.12	34.93	0.93638	46.75	39.41	36.90	0.93164	49.25	41.72	38.87
0.94082	44.30	37.17	34.97	0.93629	46.80	39.46	36.94	0.93155	49.30	41.77	38.91
0.94073	44.35	37.21	35.01	0.93619	46.85	39.50	36.98	0.93145	49.35	41.82	38.95
0.94064	44.40	37.26	35.05	0.93610	46.90	39.55	37.02	0.93136	49.40	41.87	38.99
0.94055	44.45	37.30	35.09	0.93600	46.95	39.59	37.06	0.93126	49.43	41.91	39.03
0.94046	44.50	37.35	35.13	0.93591	47.00	39.64	37.10	0.93116	49.50	41.96	39.07
0.94037	44.55	37.39	35.17	0.93582	47.05	39.68	37.14	0.93106	49.55	42.01	39.11
0.94028	44.60	37.44	35.20	0.93573	47.10	39.73	37.18	0.93096	49.60	42.06	39.15
0.94020	44.65	37.48	35.24	0.93563	47.15	39.77	37.22	0.93086	49.65	42.10	39.19
0.94011	44.70	37.53	35.28	0.93554	47.20	39.82	37.26	0.93076	49.70	42.15	39.23
0.94002	44.75	37.57	35.32	0.93545	47.25	39.87	37.30	0.93066	49.75	42.19	39.27
0.93993	44.80	37.62	35.36	0.93536	47.30	39.92	37.34	0.93056	49.80	42.24	39.31
0.93984	44.85	37.66	35.40	0.93526	47.35	39.96	37.38	0.93046	49.85	42.29	39.35
0.93975	44.90	37.71	35.44	0.93517	47.40	40.01	37.42	0.93036	49.90	42.34	39.39
0.93966	44.95	37.75	35.48	0.93507	47.45	40.05	37.46	0.93026	49.95	42.38	39.43
0.93957	45.00	37.80	35.52	0.93498	47.50	40.10	37.49	0.93017	50.00	42.43	39.47
0.93948	45.05	37.85	35.56	0.93488	47.55	40.14	37.53	0.93007	50.05	42.47	39.51
0.93939	45.10	37.90	35.60	0.93479	47.60	40.19	37.57	0.92997	50.10	42.52	39.55
0.93931	45.15	37.94	35.64	0.93470	47.65	40.24	37.61	0.92987	50.15	42.57	39.59
0.93922	45.20	37.99	35.68	0.93461	47.70	40.29	37.65	0.92977	50.20	42.62	39.63
0.93912	45.25	38.03	35.72	0.93451	47.75	40.33	37.69	0.92967	50.25	42.66	39.67
0.93903	45.30	38.08	35.76	0.93442	47.80	40.38	37.73	0.92957	50.30	42.71	39.70
0.93894	45.35	38.12	35.80	0.93432	47.85	40.42	37.77	0.92947	50.35	42.76	39.74
0.93885	45.40	38.17	35.84	0.93423	47.90	40.47	37.81	0.92938	50.40	42.81	39.78
0.93876	45.45	38.21	35.88	0.93413	47.95	40.51	37.85	0.92928	50.45	42.85	39.82
0.93867	45.50	38.26	35.92	0.93404	48.00	40.56	37.89	0.92918	50.50	42.90	39.86
0.93858	45.55	38.30	35.96	0.93394	48.05	40.61	37.93	0.92908	50.55	42.94	39.90
0.93849	45.60	38.35	35.99	0.93385	48.10	40.66	37.97	0.92898	50.60	42.99	39.94
0.93840	45.65	38.39	36.03	0.93375	48.15	40.70	38.01	0.92888	50.65	43.04	39.98
0.93831	45.70	38.44	36.07	0.93366	48.20	40.75	38.05	0.92879	50.70	43.09	40.02
0.93822	45.75	38.49	36.11	0.93356	48.25	40.79	38.09	0.92869	50.75	43.13	40.06
0.93813	45.80	38.54	36.15	0.93347	48.30	40.84	38.13	0.92859	50.80	43.18	40.10
0.93803	45.85	38.58	36.19	0.93337	48.35	40.89	38.17	0.92849	50.85	43.23	40.14
0.93794	45.90	38.63	36.23	0.93328	48.40	40.94	38.21	0.92839	50.90	43.28	40.18
0.93785	45.95	38.67	36.27	0.93318	48.45	40.98	38.25	0.92829	50.95	43.32	40.22
0.93776	46.00	38.72	36.31	0.93308	48.50	41.03	38.29	0.92818	51.00	43.37	40.26
0.93767	46.05	38.76	36.35	0.93298	48.55	41.07	38.33	0.92808	51.05	43.42	40.30
0.93758	46.10	38.81	36.39	0.93289	48.60	41.12	38.36	0.92798	51.10	43.47	40.34
0.93749	46.15	38.85	36.43	0.93279	48.65	41.16	38.40	0.92788	51.15	43.51	40.38
0.93740	46.20	38.90	36.47	0.93270	48.70	41.21	38.44	0.92778	51.20	43.56	40.42
0.93730	46.25	38.95	36.51	0.93260	48.75	41.26	38.48	0.92768	51.25	43.60	40.46
0.93721	46.30	39.00	36.55	0.93251	48.80	41.31	38.52	0.92759	51.30	43.65	40.49
0.93712	46.35	39.04	36.59	0.93241	48.85	41.35	38.56	0.92749	51.35	43.70	40.53
0.93703	46.40	39.09	36.63	0.93232	48.90	41.40	38.60	0.92739	51.40	43.75	40.57
0.93693	46.45	39.13	36.67	0.93222	48.95	41.44	38.64	0.92729	51.45	43.79	40.61
0.93684	46.50	39.18	36.70	0.93213	49.00	41.49	38.68	0.92719	51.50	43.84	40.65
0.93675	46.55	39.22	36.74	0.93203	49.05	41.54	38.72	0.92709	51.55	43.89	40.69
0.93666	46.60	39.27	36.78	0.93194	49.10	41.59	38.76	0.92699	51.60	43.94	40 73
0.93656	46.65	39.31	36.82	0.93184	49.15	41.63	38.80	0.92689	51.65	43.98	40.77
0.93647	46.70	39.36	36.86	0.93174	49.20	41.68	38.83	0.92678	51.70	44.03	40.81

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C. / 4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.
0.92668	51.75	44.08	40.85	0.92157	54.25	46.46	42.82	0.91629	56.75	48.89	44.80
0.92658	51.80	44.13	40.89	0.92147	54.30	46.51	42.86	0.91618	56.80	48.94	44.83
0.92648	51.85	44.17	40.93	0.92137	54.35	46.56	42.90	0.91608	56.85	48.98	44.87
0.92637	51.90	44.22	40.97	0.92126	54.40	46.61	42.94	0.91597	56.90	49.03	44.91
0.92627	51.95	44.26	41.01	0.92116	54.45	46.66	42.98	0.91586	56.95	49.08	44.95
0.92617	52.00	44.31	41.05	0.92105	54.50	46.71	43.02	0.91575	57.00	49.13	44.99
0.92607	52.05	44.36	41.09	0.92095	54.55	46.75	43.06	0.91565	57.05	49.18	45.03
0.92597	52.10	44.41	41.13	0.92084	54.60	46.80	43.10	0.91554	57.10	49.23	45.07
0.92587	52.15	44.46	41.17	0.92074	54.65	46.85	43.14	0.91543	57.15	49.28	45.11
0.92577	52.20	44.51	41.20	0.92063	54.70	46.90	43.18	0.91532	57.20	49.33	45.15
0.92567	52.25	44.55	41.24	0.92053	54.75	46.94	43.22	0.91521	57.25	49.38	45.19
0.92557	52.30	44.60	41.28	0.92042	54.80	46.99	43.26	0.91510	57.30	49.43	45.23
0.92547	52.35	44.65	41.32	0.92032	54.85	47.04	43.30	0.91500	57.35	49.47	45.27
0.92537	52.40	44.70	41.36	0.92021	54.90	47.09	43.34	0.91489	57.40	49.52	45.31
0.92527	52.45	44.74	41.40	0.92011	54.95	47.14	43.38	0.91478	57.45	49.57	45.35
0.92516	52.50	44.79	41.44	0.92000	55.00	47.19	43.42	0.91467	57.50	49.62	45.39
0.92506	52.55	44.84	41.48	0.91990	55.05	47.24	43.46	0.91457	57.55	49.67	45.43
0.92496	52.60	44.89	41.52	0.91979	55.10	47.29	43.49	0.91446	57.60	49.72	45.47
0.92486	52.65	44.93	41.56	0.91969	55.15	47.33	43.53	0.91435	57.65	49.77	45.51
0.92476	52.70	44.98	41.60	0.91958	55.20	47.38	43.57	0.91424	57.70	49.82	45.55
0.92466	52.75	45.03	41.64	0.91948	55.25	47.43	43.61	0.91414	57.75	49.87	45.59
0.92455	52.80	45.08	41.68	0.91937	55.30	47.48	43.65	0.91403	57.80	49.92	45.63
0.92445	52.85	45.12	41.72	0.91927	55.35	47.53	43.69	0.91392	57.85	49.96	45.67
0.92434	52.90	45.17	41.76	0.91916	55.40	47.58	43.73	0.91381	57.90	50.01	45.70
0.92424	52.95	45.22	41.80	0.91906	55.45	47.62	43.77	0.91370	57.95	50.06	45.74
0.92414	53.00	45.27	41.83	0.91895	55.50	47.67	43.81	0.91359	58.00	50.11	45.78
0.92404	53.05	45.31	41.87	0.91885	55.55	47.72	43.85	0.91348	58.05	50.16	45.82
0.92394	53.10	45.36	41.91	0.91874	55.60	47.77	43.89	0.91337	58.10	50.21	45.86
0.92384	53.15	45.41	41.95	0.91864	55.65	47.82	43.93	0.91326	58.15	50.26	45.90
0.92373	53.20	45.46	41.99	0.91853	55.70	47.87	43.97	0.91315	58.20	50.31	45.94
0.92363	53.25	45.51	42.03	0.91842	55.75	47.91	44.01	0.91304	58.25	50.36	45.98
0.92353	53.30	45.56	42.07	0.91831	55.80	47.96	44.04	0.91293	58.30	50.41	46.02
0.92343	53.35	45.60	42.11	0.91821	55.85	48.01	44.08	0.91282	58.35	50.46	46.06
0.92332	53.40	45.65	42.15	0.91810	55.90	48.06	44.12	0.91271	58.40	50.51	46.10
0.92322	53.45	45.70	42.19	0.91800	55.95	48.11	44.16	0.91261	58.45	50.55	46.14
0.92312	53.50	45.75	42.23	0.91789	56.00	48.16	44.20	0.91250	58.50	50.60	46.17
0.92302	53.55	45.79	42.27	0.91779	56.05	48.20	44.24	0.91239	58.55	50.65	46.21
0.92291	53.60	45.84	42.31	0.91768	56.10	48.25	44.28	0.91228	58.60	50.70	46.25
0.92281	53.65	45.89	42.35	0.91758	56.15	48.30	44.32	0.91217	58.65	50.75	46.29
0.92271	53.70	45.94	42.39	0.91747	56.20	48.35	44.36	0.91206	58.70	50.80	46.33
0.92261	53.75	45.98	42.43	0.91736	56.25	48.40	44.40	0.91194	58.75	50.85	46.37
0.92250	53.80	46.03	42.47	0.91725	56.30	48.45	44.44	0.91183	58.80	50.90	46.41
0.92240	53.85	46.08	42.51	0.91715	56.35	48.50	44.48	0.91171	58.85	50.95	46.45
0.92230	53.90	46.13	42.55	0.91704	56.40	48.55	44.52	0.91160	58.90	51.00	46.49
0.92220	53.95	46.18	42.59	0.91694	56.45	48.59	44.56	0.91149	58.95	51.05	46.53
0.92209	54.00	46.23	42.62	0.91683	56.50	48.64	44.60	0.91138	59.00	51.10	46.57
0.92199	54.05	46.27	42.66	0.91672	56.55	48.69	44.64	0.91127	59.05	51.15	46.61
0.92188	54.10	46.32	42.70	0.91661	56.60	48.74	44.68	0.91116	59.10	51.20	46.65
0.92178	54.15	46.36	42.74	0.91650	56.65	48.79	44.72	0.91104	59.15	51.25	46.69
0.92167	54.20	46.41	42.78	0.91639	56.70	48.84	44.76	0.91093	59.20	51.30	46.73

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.	Specific gravity 20°C./4°	Per cent by volume at 20°C.	Per cent by weight	Grams per 100 cc.
0.91082	59.25	51.35	46.77	0.90520	61.75	53.85	48.74	0.89942	64.25	56.39	50.72
0.91071	59.30	51.40	46.81	0.90509	61.80	53.90	48.78	0.89930	64.30	56.44	50.76
0.91060	59.35	51.45	46.85	0.90497	61.85	53.95	48.82	0.89918	64.35	56.49	50.80
0.91049	59.40	51.50	46.89	0.90486	61.90	54.00	48.86	0.89907	64.40	56.54	50.83
0.91038	59.45	51.55	46.93	0.90474	61.95	54.05	48.90	0.89895	64.45	56.59	50.87
0.91027	59.50	51.60	46.97	0.90463	62.00	54.10	48.94	0.89884	64.50	56.64	50.91
0.91016	59.55	51.65	47.01	0.90451	62.05	54.15	48.98	0.89872	64.55	56.70	50.95
0.91005	59.60	51.70	47.05	0.90440	62.10	54.20	49.02	0.89861	64.60	56.75	50.99
0.90993	59.65	51.74	47.09	0.90428	62.15	54.25	49.06	0.89849	64.65	56.80	51.03
0.90982	59.70	51.79	47.12	0.90417	62.20	54.30	49.10	0.89837	64.70	56.85	51.07
0.90971	59.75	51.84	47.16	0.90406	62.25	54.35	49.14	0.89825	64.75	56.90	51.11
0.90960	59.80	51.89	47.20	0.90395	62.30	54.40	49.18	0.89814	64.80	56.95	51.15
0.90949	59.85	51.94	47.24	0.90383	62.35	54.45	49.22	0.89802	64.85	57.00	51.19
0.90938	59.90	51.99	47.28	0.90372	62.40	54.50	49.25	0.89791	64.90	57.05	51.23
0.90926	59.95	52.04	47.32	0.90360	62.45	54.55	49.29	0.89779	64.95	57.11	51.27
0.90915	60.00	52.09	47.36	0.90349	62.50	54.60	49.33	0.89767	65.00	57.16	51.31
0.90904	60.05	52.14	47.40	0.90337	62.55	54.66	49.37	0.89755	65.05	57.21	51.35
0.90893	60.10	52.19	47.44	0.90326	62.60	54.71	49.41	0.89744	65.10	57.26	51.39
0.90882	60.15	52.24	47.48	0.90314	62.65	54.76	49.45	0.89732	65.15	57.31	51.43
0.90871	60.20	52.29	47.52	0.90302	62.70	54.81	49.49	0.89720	65.20	57.36	51.47
0.90859	60.25	52.34	47.56	0.90290	62.75	54.86	49.53	0.89708	65.25	57.41	51.51
0.90848	60.30	52.39	47.60	0.90279	62.80	54.91	49.57	0.89696	65.30	57.46	51.55
0.90837	60.35	52.44	47.64	0.90267	62.85	54.96	49.61	0.89684	65.35	57.52	51.59
0.90826	60.40	52.49	47.68	0.90256	62.90	55.01	49.65	0.89672	65.40	57.57	51.63
0.90814	60.45	52.54	47.72	0.90244	62.95	55.06	49.69	0.89660	65.45	57.62	51.67
0.90803	60.50	52.59	47.76	0.90233	63.00	55.11	49.73	0.89649	65.50	57.67	51.71
0.90792	60.55	52.64	47.80	0.90221	63.05	55.16	49.77	0.89637	65.55	57.72	51.75
0.90781	60.60	52.69	47.84	0.90210	63.10	55.21	49.81	0.89626	65.60	57.77	51.78
0.90769	60.65	52.74	47.88	0.90198	63.15	55.26	49.85	0.89614	65.65	57.83	51.82
0.90758	60.70	52.79	47.91	0.90187	63.20	55.31	49.89	0.89602	65.70	57.88	51.86
0.90747	60.75	52.84	47.95	0.90175	63.25	55.37	49.93	0.89590	65.75	57.93	51.90
0.90736	60.80	52.89	47.99	0.90163	63.30	55.42	49.97	0.89578	65.80	57.98	51.94
0.90724	60.85	52.94	48.03	0.90151	63.35	55.47	50.01	0.89566	65.85	58.04	51.98
0.90713	60.90	52.99	48.07	0.90140	63.40	55.52	50.04	0.89554	65.90	58.09	52.02
0.90701	60.95	53.04	48.11	0.90128	63.45	55.57	50.08	0.89542	65.95	58.14	52.06
0.90690	61.00	53.09	48.15	0.90117	63.50	55.62	50.12	0.89531	66.00	58.19	52.10
0.90678	61.05	53.14	48.19	0.90105	63.55	55.67	50.16	0.89519	66.05	58.24	52.14
0.90667	61.10	53.19	48.23	0.90094	63.60	55.72	50.20	0.89507	66.10	58.29	52.18
0.90656	61.15	53.24	48.27	0.90082	63.65	55.77	50.24	0.89495	66.15	58.35	52.22
0.90645	61.20	53.29	48.31	0.90070	63.70	55.82	50.28	0.89483	66.20	58.40	52.26
0.90633	61.25	53.34	48.35	0.90059	63.75	55.88	50.32	0.89471	66.25	58.45	52.30
0.90622	61.30	53.39	48.39	0.90048	63.80	55.93	50.36	0.89459	66.30	58.50	52.33
0.90610	61.35	53.44	48.43	0.90036	63.85	55.98	50.40	0.89447	66.35	58.55	52.37
0.90599	61.40	53.49	48.47	0.90025	63.90	56.03	50.44	0.89435	66.40	58.60	52.41
0.90588	61.45	53.55	48.51	0.90013	63.95	56.08	50.48	0.89423	66.45	58.66	52.45
0.90577	61.50	53.60	48.55	0.90001	64.00	56.13	50.52	0.89411	66.50	58.71	52.49
0.90565	61.55	53.65	48.59	0.89989	64.05	56.18	50.56	0.89399	66.55	58.76	52.53
0.90554	61.60	53.70	48.62	0.89978	64.10	56.23	50.60	0.89387	66.60	58.81	52.57
0.90543	61.65	53.75	48.66	0.89966	64.15	56.29	50.64	0.89375	66.65	58.87	52.61
0.90532	61.70	53.80	48.70	0.89954	64.20	56.34	50.68	0.89363	66.70	58.92	52.65

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.89351	66.75	58.97	52.69	0.88744	69.25	61.60	54.66	0.88120	71.75	64.27	56.64
0.89339	66.80	59.02	52.73	0.88732	69.30	61.65	54.70	0.88107	71.80	64.32	56.68
0.89327	66.85	59.07	52.77	0.88720	69.35	61.70	54.74	0.88094	71.85	64.38	56.72
0.89315	66.90	59.12	52.81	0.88707	69.40	61.75	54.78	0.88081	71.90	64.43	56.75
0.89303	66.95	59.18	52.85	0.88695	69.45	61.81	54.82	0.88069	71.95	64.49	56.79
0.89291	67.00	59.23	52.89	0.88682	69.50	61.86	54.86	0.88056	72.00	64.54	56.83
0.89279	67.05	59.28	52.93	0.88670	69.55	61.92	54.90	0.88044	72.05	64.60	56.87
0.89267	67.10	59.33	52.97	0.88658	69.60	61.97	54.94	0.88031	72.10	64.65	56.91
0.89255	67.15	59.39	53.01	0.88646	69.65	62.02	54.98	0.88018	72.15	64.71	56.95
0.89243	67.20	59.44	53.04	0.88633	69.70	62.07	55.02	0.88005	72.20	64.76	56.99
0.89231	67.25	59.49	53.08	0.88621	69.75	62.13	55.06	0.87993	72.25	64.82	57.03
0.89219	67.30	59.54	53.12	0.88608	69.80	62.18	55.10	0.87980	72.30	64.87	57.07
0.89207	67.35	59.60	53.16	0.88596	69.85	62.24	55.14	0.87967	72.35	64.93	57.11
0.89195	67.40	59.65	53.20	0.88583	69.90	62.29	55.18	0.87954	72.40	64.98	57.15
0.89183	67.45	59.70	53.24	0.88571	69.95	62.34	55.22	0.87942	72.45	65.03	57.19
0.89171	67.50	59.75	53.28	0.88558	70.00	62.39	55.25	0.87929	72.50	65.08	57.23
0.89159	67.55	59.81	53.32	0.88546	70.05	62.45	55.29	0.87916	72.55	65.14	57.27
0.89147	67.60	59.86	53.36	0.88533	70.10	62.50	55.33	0.87903	72.60	65.19	57.31
0.89135	67.65	59.91	53.40	0.88521	70.15	62.56	55.37	0.87891	72.65	65.25	57.35
0.89122	67.70	59.96	53.44	0.88508	70.20	62.61	55.41	0.87878	72.70	65.30	57.38
0.89110	67.75	60.02	53.48	0.88496	70.25	62.66	55.45	0.87865	72.75	65.36	57.42
0.89098	67.80	60.07	53.52	0.88484	70.30	62.71	55.49	0.87852	72.80	65.41	57.46
0.89086	67.85	60.12	53.56	0.88472	70.35	62.77	55.53	0.87839	72.85	65.47	57.50
0.89074	67.90	60.17	53.60	0.88459	70.40	62.82	55.57	0.87826	72.90	65.52	57.54
0.89062	67.95	60.23	53.64	0.88447	70.45	62.87	55.61	0.87813	72.95	65.58	57.58
0.89050	68.00	60.28	53.68	0.88434	70.50	62.92	55.65	0.87800	73.00	65.63	57.62
0.89038	68.05	60.33	53.72	0.88422	70.55	62.98	55.69	0.87788	73.05	65.68	57.66
0.89026	68.10	60.38	53.75	0.88409	70.60	63.03	55.73	0.87775	73.10	65.74	57.70
0.89014	68.15	60.44	53.79	0.88397	70.65	63.09	55.77	0.87762	73.15	65.80	57.74
0.89001	68.20	60.49	53.83	0.88384	70.70	63.14	55.81	0.87749	73.20	65.85	57.77
0.88989	68.25	60.54	53.87	0.88372	70.75	63.20	55.85	0.87737	73.25	65.91	57.82
0.88977	68.30	60.59	53.91	0.88359	70.80	63.25	55.89	0.87724	73.30	65.96	57.86
0.88965	68.35	60.65	53.95	0.88347	70.85	63.31	55.93	0.87711	73.35	66.02	57.90
0.88952	68.40	60.70	53.99	0.88334	70.90	63.36	55.97	0.87698	73.40	66.07	57.94
0.88940	68.45	60.75	54.03	0.88322	70.95	63.41	56.01	0.87685	73.45	66.13	57.98
0.88928	68.50	60.80	54.07	0.88309	71.00	63.46	56.04	0.87672	73.50	66.18	58.02
0.88916	68.55	60.86	54.11	0.88297	71.05	63.52	56.08	0.87659	73.55	66.23	58.06
0.88904	68.60	60.91	54.15	0.88284	71.10	63.57	56.12	0.87646	73.60	66.28	58.10
0.88892	68.65	60.96	54.19	0.88272	71.15	63.63	56.16	0.87633	73.65	66.34	58.14
0.88879	68.70	61.01	54.23	0.88259	71.20	63.68	56.20	0.87620	73.70	66.39	58.17
0.88867	68.75	61.07	54.27	0.88246	71.25	63.74	56.24	0.87607	73.75	66.45	58.21
0.88854	68.80	61.12	54.31	0.88233	71.30	63.79	56.28	0.87594	73.80	66.50	58.25
0.88842	68.85	61.17	54.35	0.88221	71.35	63.84	56.32	0.87581	73.85	66.56	58.29
0.88830	68.90	61.22	54.39	0.88208	71.40	63.89	56.36	0.87568	73.90	66.61	58.33
0.88818	68.95	61.28	54.43	0.88196	71.45	63.95	56.40	0.87555	73.95	66.67	58.37
0.88805	69.00	61.33	54.47	0.88183	71.50	64.00	56.44	0.87542	74.00	66.72	58.41
0.88793	69.05	61.39	54.51	0.88171	71.55	64.06	56.48	0.87529	74.05	66.78	58.45
0.88781	69.10	61.44	54.54	0.88158	71.60	64.11	56.52	0.87516	74.10	66.83	58.49
0.88769	69.15	61.49	54.58	0.88145	71.65	64.17	56.56	0.87504	74.15	66.89	58.53
0.88756	69.20	61.54	54.62	0.88132	71.70	64.22	56.60	0.87491	74.20	66.94	58.57

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.87478	74.25	67.00	58.61	0.86818	76.75	69.78	60.58	0.86137	79.25	72.63	62.56
0.87465	74.30	67.05	58.65	0.86805	76.80	69.84	60.62	0.86124	79.30	72.68	62.60
0.87452	74.35	67.11	58.69	0.86791	76.85	69.90	60.66	0.86110	79.35	72.74	62.64
0.87439	74.40	67.16	58.73	0.86778	76.90	69.95	60.70	0.86096	79.40	72.80	62.67
0.87426	74.45	67.22	58.77	0.86764	76.95	70.01	60.74	0.86082	79.45	72.86	62.71
0.87413	74.50	67.27	58.81	0.86751	77.00	70.06	60.78	0.86069	79.50	72.91	62.75
0.87400	74.55	67.33	58.85	0.86737	77.05	70.12	60.82	0.86055	79.55	72.97	62.79
0.87387	74.60	67.38	58.89	0.86724	77.10	70.18	60.86	0.86041	79.60	73.03	62.83
0.87373	74.65	67.44	58.93	0.86710	77.15	70.24	60.90	0.86027	79.65	73.09	62.87
0.87360	74.70	67.49	58.97	0.86697	77.20	70.29	60.94	0.86013	79.70	73.14	62.91
0.87347	74.75	67.55	59.01	0.86684	77.25	70.35	60.98	0.85999	79.75	73.20	62.95
0.87334	74.80	67.61	59.04	0.86671	77.30	70.40	61.02	0.85984	79.80	73.26	62.99
0.87321	74.85	67.67	59.08	0.86657	77.35	70.46	61.06	0.85970	79.85	73.32	63.03
0.87308	74.90	67.72	59.12	0.86644	77.40	70.51	61.10	0.85956	79.90	73.37	63.07
0.87295	74.95	67.78	59.16	0.86630	77.45	70.57	61.14	0.85942	79.95	73.43	63.11
0.87282	75.00	67.83	59.20	0.86617	77.50	70.63	61.18	0.85928	80.00	73.49	63.15
0.87269	75.05	67.89	59.24	0.86603	77.55	70.69	61.22	0.85914	80.05	73.55	63.19
0.87256	75.10	67.94	59.28	0.86589	77.60	70.74	61.25	0.85901	80.10	73.60	63.23
0.87243	75.15	68.00	59.32	0.86575	77.65	70.80	61.29	0.85887	80.15	73.66	63.27
0.87230	75.20	68.05	59.36	0.86562	77.70	70.85	61.33	0.85873	80.20	73.72	63.30
0.87217	75.25	68.11	59.40	0.86548	77.75	70.91	61.37	0.85859	80.25	73.78	63.34
0.87204	75.30	68.16	59.44	0.86535	77.80	70.97	61.41	0.85846	80.30	73.83	63.38
0.87190	75.35	68.22	59.48	0.86521	77.85	71.03	61.45	0.85832	80.35	73.89	63.42
0.87177	75.40	68.27	59.52	0.86508	77.90	71.08	61.49	0.85818	80.40	73.95	63.46
0.87164	75.45	68.33	59.56	0.86494	77.95	71.14	61.53	0.85804	80.45	74.01	63.50
0.87151	75.50	68.38	59.60	0.86480	78.00	71.19	61.57	0.85789	80.50	74.06	63.54
0.87138	75.55	68.44	59.64	0.86466	78.05	71.25	61.61	0.85775	80.55	74.12	63.58
0.87125	75.60	68.49	59.67	0.86453	78.10	71.31	61.65	0.85761	80.60	74.18	63.62
0.87111	75.65	68.55	59.71	0.86439	78.15	71.37	61.69	0.85747	80.65	74.24	63.66
0.87098	75.70	68.60	59.75	0.86426	78.20	71.42	61.73	0.85733	80.70	74.30	63.70
0.87084	75.75	68.66	59.79	0.86412	78.25	71.48	61.77	0.85719	80.75	74.36	63.74
0.87071	75.80	68.72	59.83	0.86399	78.30	71.54	61.81	0.85707	80.80	74.42	63.78
0.87058	75.85	68.78	59.87	0.86385	78.35	71.60	61.85	0.85691	80.85	74.48	63.82
0.87045	75.90	68.83	59.91	0.86371	78.40	71.65	61.88	0.85677	80.90	74.53	63.86
0.87032	75.95	68.89	59.95	0.86357	78.45	71.71	61.92	0.85663	80.95	74.59	63.90
0.87019	76.00	68.94	59.99	0.86344	78.50	71.76	61.96	0.85648	81.00	74.65	63.94
0.87005	76.05	69.00	60.03	0.86330	78.55	71.82	62.00	0.85634	81.05	74.71	63.98
0.86992	76.10	69.05	60.07	0.86316	78.60	71.88	62.04	0.85620	81.10	74.77	64.02
0.86979	76.15	69.11	60.11	0.86302	78.65	71.94	62.08	0.85606	81.15	74.83	64.06
0.86966	76.20	69.16	60.15	0.86289	78.70	71.99	62.12	0.85592	81.20	74.88	64.09
0.86952	76.25	69.22	60.19	0.86275	78.75	72.05	62.16	0.85578	81.25	74.94	64.13
0.86939	76.30	69.27	60.23	0.86261	78.80	72.11	62.20	0.85564	81.30	75.00	64.17
0.86925	76.35	69.33	60.27	0.86247	78.85	72.17	62.24	0.85550	81.35	75.06	64.21
0.86912	76.40	69.39	60.31	0.86234	78.90	72.22	62.28	0.85536	81.40	75.12	64.25
0.86898	76.45	69.45	60.35	0.86220	78.95	72.28	62.32	0.85522	81.45	75.18	64.29
0.86885	76.50	69.50	60.39	0.86206	79.00	72.34	62.36	0.85507	81.50	75.24	64.33
0.86872	76.55	69.56	60.43	0.86192	79.05	72.40	62.40	0.85493	81.55	75.30	64.37
0.86859	76.60	69.61	60.47	0.86179	79.10	72.45	62.44	0.85478	81.60	75.35	64.41
0.86845	76.65	69.67	60.51	0.86165	79.15	72.51	62.48	0.85464	81.65	75.41	64.45
0.86832	76.70	69.72	60.54	0.86151	79.20	72.57	62.52	0.85450	81.70	75.47	64.49

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C. 4°	Alcohol			Specific gravity 20°C. 4°	Alcohol			Specific gravity 20°C. 4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.85436	81.75	75.53	64.53	0.84713	84.25	78.50	66.50	0.83957	86.75	81.56	68.48
0.85422	81.80	75.59	64.57	0.84698	84.30	78.56	66.54	0.83942	86.80	81.62	68.52
0.85408	81.85	75.65	64.61	0.84683	84.35	78.62	66.58	0.83927	86.85	81.68	68.56
0.85393	81.90	75.71	64.65	0.84668	84.40	78.68	66.62	0.83912	86.90	81.75	68.60
0.85379	81.95	75.77	64.69	0.84654	84.45	78.74	66.66	0.83896	86.95	81.81	68.64
0.85364	82.00	75.82	64.73	0.84639	84.50	78.80	66.70	0.83881	87.00	81.87	68.68
0.85350	82.05	75.88	64.77	0.84624	84.55	78.86	66.74	0.83865	87.05	81.93	68.72
0.85336	82.10	75.94	64.81	0.84609	84.60	78.93	66.78	0.83850	87.10	81.99	68.76
0.85322	82.15	76.00	64.85	0.84594	84.65	78.99	66.82	0.83834	87.15	82.05	68.80
0.85307	82.20	76.06	64.88	0.84579	84.70	79.05	66.86	0.83818	87.20	82.12	68.84
0.85293	82.25	76.12	64.92	0.84564	84.75	79.11	66.90	0.83802	87.25	82.18	68.88
0.85279	82.30	76.18	64.96	0.84549	84.80	79.17	66.94	0.83787	87.30	82.24	68.91
0.85265	82.35	76.24	65.00	0.84534	84.85	79.23	66.98	0.83771	87.35	82.30	68.95
0.85250	82.40	76.30	65.04	0.84519	84.90	79.29	67.02	0.83756	87.40	82.37	68.99
0.85236	82.45	76.36	65.08	0.84504	84.95	79.35	67.06	0.83740	87.45	82.43	69.03
0.85222	82.50	76.41	65.12	0.84489	85.00	79.41	67.09	0.83725	87.50	82.49	69.07
0.85207	82.55	76.47	65.16	0.84474	85.05	79.47	67.13	0.83709	87.55	82.55	69.11
0.85192	82.60	76.53	65.20	0.84459	85.10	79.53	67.17	0.83694	87.60	82.62	69.15
0.85178	82.65	76.59	65.24	0.84444	85.15	79.59	67.21	0.83678	87.65	82.68	69.19
0.85164	82.70	76.65	65.28	0.84429	85.20	79.65	67.25	0.83663	87.70	82.74	69.23
0.85150	82.75	76.71	65.32	0.84414	85.25	79.71	67.29	0.83647	87.75	82.80	69.27
0.85135	82.80	76.77	65.36	0.84399	85.30	79.78	67.33	0.83632	87.80	82.87	69.30
0.85121	82.85	76.83	65.40	0.84384	85.35	79.84	67.37	0.83616	87.85	82.93	69.34
0.85106	82.90	76.89	65.44	0.84369	85.40	79.90	67.41	0.83601	87.90	82.99	69.38
0.85092	82.95	76.95	65.48	0.84354	85.45	79.96	67.45	0.83585	87.95	83.05	69.42
0.85077	83.00	77.01	65.51	0.84339	85.50	80.02	67.49	0.83569	88.00	83.12	69.46
0.85063	83.05	77.07	65.55	0.84323	85.55	80.08	67.53	0.83553	88.05	83.18	69.50
0.85049	83.10	77.13	65.59	0.84308	85.60	80.14	67.57	0.83537	88.10	83.25	69.54
0.85035	83.15	77.19	65.63	0.84293	85.65	80.20	67.61	0.83521	88.15	83.31	69.58
0.85020	83.20	77.24	65.67	0.84278	85.70	80.27	67.65	0.83505	88.20	83.37	69.62
0.85006	83.25	77.30	65.71	0.84263	85.75	80.33	67.69	0.83489	88.25	83.43	69.66
0.84991	83.30	77.36	65.75	0.84248	85.80	80.39	67.73	0.83473	88.30	83.50	69.70
0.84977	83.35	77.42	65.79	0.84233	85.85	80.45	67.77	0.83457	88.35	83.56	69.74
0.84962	83.40	77.48	65.83	0.84218	85.90	80.51	67.80	0.83442	88.40	83.63	69.78
0.84948	83.45	77.54	65.87	0.84203	85.95	80.57	67.84	0.83426	88.45	83.69	69.82
0.84933	83.50	77.60	65.91	0.84188	86.00	80.63	67.88	0.83410	88.50	83.75	69.86
0.84918	83.55	77.66	65.95	0.84172	86.05	80.69	67.92	0.83394	88.55	83.81	69.90
0.84903	83.60	77.72	65.99	0.84157	86.10	80.76	67.96	0.83379	88.60	83.88	69.94
0.84889	83.65	77.78	66.03	0.84141	86.15	80.82	68.00	0.83363	88.65	83.94	69.98
0.84874	83.70	77.84	66.07	0.84126	86.20	80.88	68.04	0.83347	88.70	84.00	70.01
0.84859	83.75	77.90	66.11	0.84110	86.25	80.94	68.08	0.83331	88.75	84.06	70.05
0.84844	83.80	77.96	66.15	0.84095	86.30	81.00	68.12	0.83315	88.80	84.13	70.09
0.84830	83.85	78.02	66.19	0.84080	86.35	81.06	68.16	0.83299	88.85	84.19	70.13
0.84815	83.90	78.08	66.23	0.84065	86.40	81.13	68.20	0.83283	88.90	84.26	70.17
0.84801	83.95	78.14	66.27	0.84049	86.45	81.19	68.24	0.83267	88.95	84.32	70.21
0.84786	84.00	78.20	66.30	0.84034	86.50	81.25	68.28	0.83251	89.00	84.39	70.25
0.84772	84.05	78.26	66.34	0.84018	86.55	81.31	68 32	0.83235	89.05	84.45	70.29
0.84757	84.10	78.32	66.38	0.84003	86.60	81.37	68.36	0.83219	89.10	84.51	70.33
0.84742	84.15	78.38	66.42	0.83987	86.65	81.43	68.40	0.83203	89.15	84.57	70.37
0.84727	84.20	78.44	66.46	0.83972	86.70	81.50	68.44	0.83186	89.20	84.64	70.41

TABLE 82.—ALCOHOL TABLE.—(*Continued*)

Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol			Specific gravity 20°C./4°	Alcohol		
	Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20°C.	Per cent. by weight	Grams per 100 cc.
0.83170	89.25	84.70	70.45	0.82332	91.75	87.96	72.42	0.81432	94.25	91.36	74.40
0.83154	89.30	84.77	70.49	0.82315	91.80	88.03	72.46	0.81413	94.30	91.43	74.44
0.83138	89.35	84.83	70.53	0.82298	91.85	88.09	72.50	0.81394	94.35	91.50	74.48
0.83121	89.40	84.90	70.57	0.82281	91.90	88.16	72.54	0.81375	94.40	91.57	74.52
0.83105	89.45	84.96	70.61	0.82263	91.95	88.22	72.58	0.81356	94.45	91.64	74.56
0.83089	89.50	85.03	70.65	0.82246	92.00	88.29	72.62	0.81337	94.50	91.71	74.59
0.83073	89.55	85.09	70.69	0.82229	92.05	88.36	72.66	0.81318	94.55	91.78	74.63
0.83056	89.60	85.15	70.72	0.82212	92.10	88.43	72.70	0.81299	94.60	91.85	74.67
0.83040	89.65	85.21	70.76	0.82194	92.15	88.49	72.74	0.81280	94.65	91.92	74.71
0.83024	89.70	85.28	70.80	0.82177	92.20	88.56	72.78	0.81260	94.70	91.99	74.75
0.83008	89.75	85.34	70.84	0.82159	92.25	88.63	72.82	0.81241	94.75	92.06	74.79
0.82991	89.80	85.41	70.88	0.82141	92.30	88.70	72.86	0.81222	94.80	92.13	74.83
0.82975	89.85	85.47	70.92	0.82123	92.35	88.76	72.90	0.81202	94.85	92.20	74.87
0.82958	89.90	85.54	70.96	0.82106	92.40	88.83	72.94	0.81183	94.90	92.27	74.91
0.82942	89.95	85.60	71.00	0.82088	92.45	88.89	72.98	0.81163	94.95	92.34	74.95
0.82925	90.00	85.67	71.04	0.82071	92.50	88.96	73.02	0.81144	95.00	92.41	74.99
0.82909	90.05	85.73	71.08	0.82053	92.55	89.03	73.06	0.81124	95.05	92.48	75.03
0.82892	90.10	85.80	71.12	0.82035	92.60	89.10	73.09	0.81105	95.10	92.55	75.07
0.82876	90.15	85.86	71.16	0.82017	92.65	89.16	73.13	0.81086	95.15	92.63	75.11
0.82859	90.20	85.93	71.20	0.82000	92.70	89.23	73.17	0.81067	95.20	92.70	75.15
0.82843	90.25	85.99	71.24	0.81982	92.75	89.30	73.21	0.81047	95.25	92.77	75.19
0.82826	90.30	86.06	71.28	0.81964	92.80	89.37	73.25	0.81028	95.30	92.84	75.23
0.82810	90.35	86.12	71.32	0.81946	92.85	89.43	73.29	0.81008	95.35	92.91	75.27
0.82793	90.40	86.19	71.36	0.81929	92.90	89.50	73.33	0.80988	95.40	92.98	75.30
0.82776	90.45	86.25	71.40	0.81911	92.95	89.57	73.37	0.80968	95.45	93.05	75.34
0.82759	90.50	86.32	71.44	0.81893	93.00	89.64	73.41	0.80949	95.50	93.12	75.38
0.82742	90.55	86.38	71.48	0.81875	93.05	89.71	73.45	0.80929	95.55	93.20	75.42
0.82725	90.60	86.45	71.52	0.81856	93.10	89.78	73.49	0.80909	95.60	93.27	75.46
0.82708	90.65	86.51	71.56	0.81838	93.15	89.84	73.53	0.80889	95.65	93.34	75.50
0.82691	90.70	86.58	71.59	0.81821	93.20	89.91	73.57	0.80869	95.70	93.41	75.54
0.82674	90.75	86.64	71.63	0.81803	93.25	89.98	73.61	0.80849	95.75	93.48	75.58
0.82657	90.80	86.71	71.67	0.81784	93.30	90.05	73.65	0.80829	95.80	93.55	75.62
0.82640	90.85	86.77	71.71	0.81766	93.35	90.12	73.69	0.80809	95.85	93.63	75.66
0.82624	90.90	86.84	71.75	0.81748	93.40	90.19	73.72	0.80789	95.90	93.70	75.70
0.82607	90.95	86.90	71.79	0.81730	93.45	90.25	73.76	0.80769	95.95	93.77	75.74
0.82590	91.00	86.97	71.83	0.81711	93.50	90.32	73.80	0.80749	96.00	93.84	75.78
0.82573	91.05	87.03	71.87	0.81693	93.55	90.39	73.84	0.80729	96.05	93.92	75.82
0.82556	91.10	87.10	71.91	0.81675	93.60	90.46	73.88	0.80709	96.10	93.99	75.86
0.82539	91.15	87.17	71.95	0.81657	93.65	90.53	73.92	0.80689	96.15	94.06	75.90
0.82522	91.20	87.24	71.99	0.81638	93.70	90.60	73.96	0.80668	96.20	94.13	75.94
0.82505	91.25	87.30	72.03	0.81620	93.75	90.67	74.00	0.80648	96.25	94.21	75.98
0.82488	91.30	87.37	72.07	0.81601	93.80	90.74	74.04	0.80627	96.30	94.28	76.01
0.82470	91.35	87.43	72.11	0.81582	93.85	90.80	74.08	0.80607	96.35	94.35	76.05
0.82453	91.40	87.50	72.15	0.81563	93.90	90.87	74.12	0.80586	96.40	94.42	76.09
0.82436	91.45	87.56	72.19	0.81545	93.95	90.94	74.16	0.80566	96.45	94.50	76.13
0.82419	91.50	87.63	72.23	0.81526	94.00	91.01	74.20	0.80545	96.50	94.57	76.17
0.82401	91.55	87.69	72.27	0.81507	94.05	91.08	74.24	0.80525	96.55	94.65	76.21
0.82384	91.60	87.76	72.30	0.81488	94.10	91.15	74.28	0.80504	96.60	94.72	76.25
0.82367	91.65	87.83	72.34	0.81469	94.15	91.22	74.32	0.80483	96.65	94.79	76.29
0.82350	91.70	87.90	72.38	0.81450	94.20	91.29	74.36	0.80462	96.70	94.86	76.33

TABLE 82.—ALCOHOL TABLE.—(Concluded)

Specific gravity 20°C. 4°	Alcohol			Specific Gravity 20°C. 4°	Alcohol			Specific Gravity 20°C. 4°	Alcohol		
	Per cent. by volume at 20° C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20° C.	Per cent. by weight	Grams per 100 cc.		Per cent. by volume at 20° C.	Per cent. by weight	Grams per 100 cc.
0.80442	96.75	94.94	76.37	0.79900	98.00	96.82	77.36	0.79311	99.25	98.78	78.34
0.80421	96.80	95.01	76.41	0.79878	98.05	96.90	77.40	0.79286	99.30	98.86	78.38
0.80400	96.85	95.09	76.45	0.79855	98.10	96.97	77.43	0.79262	99.35	98.94	78.42
0.80379	96.90	95.16	76.49	0.79832	98.15	97.05	77.47	0.79237	99.40	99.02	78.46
0.80358	96.95	95.24	76.53	0.79809	98.20	97.12	77.51	0.79213	99.45	99.10	78.50
0.80337	97.00	95.31	76.57	0.79786	98.25	97.20	77.55	0.79188	99.50	99.18	78.54
0.80315	97.05	95.39	76.61	0.79763	98.30	97.28	77.59	0.79163	99.55	99.26	78.58
0.80294	97.10	95.46	76.65	0.79740	98.35	97.36	77.63	0.79138	99.60	99.34	78.62
0.80273	97.15	95.53	76.69	0.79717	98.40	97.43	77.67	0.79113	99.65	99.42	78.66
0.80252	97.20	95.60	76.72	0.79695	98.45	97.51	77.71	0.79088	99.70	99.51	78.70
0.80230	97.25	95.68	76.76	0.79672	98.50	97.59	77.75	0.79062	99.75	99.59	78.74
0.80208	97.30	95.75	76.80	0.79648	98.55	97.67	77.79	0.79037	99.80	99.67	78.78
0.80186	97.35	95.83	76.84	0.79625	98.60	97.75	77.83	0.79011	99.85	99.75	78.82
0.80164	97.40	95.91	76.88	0.79601	98.65	97.83	77.87	0.78986	99.90	99.83	78.86
0.80143	97.45	95.98	76.92	0.79577	98.70	97.90	77.91	0.78960	99.95	99.91	78.90
0.80122	97.50	96.05	76.96	0.79553	98.75	97.98	77.95	0.78934	100.00	100.00	78.93
0.80100	97.55	96.13	77.00	0.79529	98.80	98.06	77.99				
0.80078	97.60	96.21	77.04	0.79505	98.85	98.14	78.03				
0.80056	97.65	96.29	77.08	0.79481	98.90	98.22	78.07				
0.80034	97.70	96.36	77.12	0.79457	98.95	98.30	78.11				
0.80012	97.75	96.44	77.16	0.79432	99.00	98.38	78.14				
0.79990	97.80	96.52	77.20	0.79408	99.05	98.46	78.18				
0.79968	97.85	96.60	77.24	0.79384	99.10	98.54	78.22				
0.79945	97.90	96.68	77.28	0.79360	99.15	98.62	78.26				
0.79923	97.95	96.75	77.32	0.79335	99.20	98.70	78.30				

distillate and the water weighings, its effect on the specific-gravity figure is very slight and can be neglected for all ordinary work.

Detection of Methyl Alcohol.—The occasional use has been reported in food products of methyl alcohol in place of the more costly ethyl alcohol. This is most likely to occur in such products as flavoring extracts or distilled liquors and might be due to the use of "denatured" alcohol rather than to the direct addition of methyl alcohol itself.

Many methods for the detection of methyl alcohol in the presence of ethyl alcohol have been proposed,[1] but it will suffice to describe only three as especially applicable in food analysis, the first two because they are rapid and require no special apparatus, the third because it is both qualitative and quantitative. Any test used should be applied to the *distillate* obtained in the determination of alcohol as previously described.

[1] For an exhaustive list see GETTLER: *J. Biol. Chem.*, **1920**, 311.

TABLE 83.[1]—REFRACTIVE INDEXES AND ZEISS IMMERSION REFRACTOMETER READINGS[2] OF ALCOHOL-WATER MIXTURES AT 20 AND 25°C.

Scale reading	Refractive index	Per cent alcohol by volume		Scale reading	Refractive index	Per cent alcohol by volume	
		20°C.	25°C.			20°C.	25°C.
13.2	1.33250		0.00	20.0	1.33513	4.32	5.29
.4	3257		0.18	.2	3520	4.47	5.44
.6	3265		0.35	.4	3528	4.61	5.58
.8	3273		0.53	.6	3536	4.75	5.72
				.8	3543	4.90	5.87
14.0	3281		0.70				
.2	3288		0.88	21.0	3551	5.04	6.02
.4	3296		1.06	.2	3559	5.19	6.16
.6	3304	0.16	1.24	.4	3566	5.33	6.30
.8	3312	0.34	1.40	.6	3574	5.47	6.44
				.8	3582	5.61	6.59
15.0	3319	0.52	1.55				
.2	3327	0.69	1.71	22.0	3590	5.76	6.73
.4	3335	0.85	1.86	.2	3597	5.90	6.87
.6	3343	1.03	2.01	.4	3605	6.05	7.01
.8	3350	1.21	2.17	.6	3613	6.19	7.16
				.8	3620	6.33	7.31
16.0	3358	1.36	2.33				
.2	3366	1.51	2.48	23.0	3628	6.47	7.45
.4	3374	1.66	2.62	.2	3636	6.61	7.59
.6	3381	1.81	2.77	.4	3643	6.75	7.73
.8	3389	1.96	2.92	.6	3651	6.90	7.87
				.8	3659	7.04	8.00
17.0	3397	2.11	3.06				
.2	3405	2.26	3.21	24.0	3666	7.18	8.14
.4	3412	2.41	3.36	.2	3674	7.32	8.28
.6	3420	2.56	3.51	.4	3682	7.46	8.42
.8	3428	2.70	3.66	.6	3689	7.60	8.55
				.8	3697	7.74	8.69
18.0	3435	2.85	3.81				
.2	3443	3.00	3.96	25.0	3705	7.88	8.84
.4	3451	3.15	4.11	.2	3712	8.01	8.98
.6	3459	3.30	4.26	.4	3720	8.14	9.12
.8	3466	3.45	4.41	.6	3728	8.28	9.26
				.8	3735	8.42	9.39
19.0	3474	3.59	4.56				
.2	3482	3.73	4.70	26.0	3743	8.55	9.53
.4	3489	3.88	4.85	.2	3751	8.69	9.67
.6	3497	4.03	5.00	.4	3758	8.82	9.81
.8	3505	4.17	5.15	.6	3766	8.96	9.95
				.8	3774	9.10	10.09

TABLE 83.[1]—REFRACTIVE INDEXES AND ZEISS IMMERSION REFRACTOMETER READINGS[2] OF ALCOHOL-WATER MIXTURES AT 20 AND 25°C.—(*Continued*)

Scale reading	Refractive index	Per cent alcohol by volume		Scale reading	Refractive index	Per cent alcohol by volume	
		20°C.	25°C.			20°C.	25°C.
27.0	1.33781	9.23	10.23	34.0	1.34048	13.86	14.98
.2	3789	9.37	10.37	.2	4056	13.99	15.11
.4	3796	9.51	10.51	.4	4063	14.12	15.25
.6	3804	9.65	10.65	.6	4071	14.25	15.38
.8	3812	9.79	10.79	.8	4078	14.37	15.51
28.0	3820	9.92	10.93	35.0	4086	14.50	15.65
.2	3827	10.06	11.06	.2	4094	14.62	15.78
.4	3835	10.19	11.20	.4	4101	14.75	15.91
.6	3842	10.32	11.33	.6	4109	14.87	16.05
.8	3850	10.46	11.47	.8	4116	15.00	16.18
29.0	3858	10.59	11.61	36.0	4124	15.13	16.31
.2	3865	10.73	11.75	.2	4131	15.25	16.44
.4	3873	10.86	11.88	.4	4139	15.38	16.56
.6	3881	10.99	12.01	.6	4146	15.51	16.69
.8	3888	11.12	12.15	.8	4154	15.63	16.82
30.0	3896	11.26	12.29	37.0	4162	15.76	16.95
.2	3904	11.38	12.42	.2	4169	15.89	17.08
.4	3911	11.51	12.56	.4	4177	16.01	17.21
.6	3919	11.64	12.70	.6	4184	16.14	17.34
.8	3926	11.78	12.84	.8	4192	16.26	17.46
31.0	3934	11.91	12.97	38.0	4199	16.39	17.59
.2	3942	12.04	13.11	.2	4207	16.51	17.72
.4	3949	12.17	13.24	.4	4215	16.64	17.85
.6	3957	12.30	13.37	.6	4222	16.76	17.97
.8	3964	12.43	13.51	.8	4230	16.89	18.10
32.0	3972	12.57	13.64	39.0	4237	17.01	18.23
.2	3980	12.70	13.77	.2	4245	17.14	18.35
.4	3987	12.83	13.91	.4	4252	17.26	18.48
.6	3995	12.96	14.04	.6	4260	17.39	18.61
.8	4002	13.09	14.17	.8	4267	17.51	18.73
33.0	4010	13.22	14.31	40.0	4275	17.63	18.86
.2	4018	13.35	14.44	.2	4282	17.76	18.99
.4	4025	13.48	14.58	.4	4290	17.88	19.11
.6	4033	13.61	14.71	.6	4298	18.01	19.24
.8	4040	13.74	14.85	.8	4305	18.13	19.37

TABLE 83.[1]—REFRACTIVE INDEXES AND ZEISS IMMERSION REFRACTOMETER READINGS[2] OF ALCOHOL-WATER MIXTURES AT 20 AND 25°C.—(*Continued*)

Scale reading	Refractive index	Per cent alcohol by volume		Scale reading	Refractive index	Per cent alcohol by volume	
		20°C.	25°C.			20°C.	25°C.
41.0	1.34313	18.25	19.49	48.0	1.34575	22.51	23.93
.2	4320	18.37	19.62	.2	4583	22.63	24.06
.4	4328	18.50	19.75	.4	4590	22.75	24.19
.6	4335	18.62	19.87	.6	4598	22.87	24.32
.8	4343	18.74	20.00	.8	4605	22.99	24.45
42.0	4350	18.87	20.13	49.0	4613	23.12	24.59
.2	4358	18.99	20.25	.2	4620	23.24	24.72
.4	4365	19.11	20.38	.4	4628	23.36	24.85
.6	4373	19.23	20.50	.6	4635	23.48	24.98
.8	4380	19.36	20.63	.8	4643	23.61	25.11
43.0	4388	19.48	20.75	50.0	4650	23.73	25.25
.2	4395	19.60	20.88	.2	4658	23.85	25.38
.4	4403	19.72	21.01	.4	4665	23.98	25.51
.6	4410	19.85	21.13	.6	4672	24.10	25.65
.8	4418	19.97	21.25	.8	4680	24.22	25.78
44.0	4426	20.09	21.38	51.0	4687	24.35	25.91
.2	4433	20.21	21.50	.2	4695	24.47	26.05
.4	4440	20.33	21.63	.4	4702	24.59	26.18
.6	4448	20.45	21.75	.6	4710	24.72	26.32
.8	4456	20.58	21.88	.8	4717	24.84	26.45
45.0	4463	20.70	22.00	52.0	4724	24.96	26.59
.2	4470	20.82	22.13	.2	4732	25.09	26.72
.4	4478	20.94	22.25	.4	4740	25.21	26.86
.6	4486	21.06	22.38	.6	4747	25.34	26.99
.8	4493	21.18	22.51	.8	4754	25.46	27.13
46.0	4500	21.30	22.64	53.0	4762	25.59	27.27
.2	4508	21.42	22.76	.2	4769	25.71	27.40
.4	4516	21.54	22.89	.4	4777	25.84	27.54
.6	4523	21.66	23.02	.6	4784	25.96	27.67
.8	4530	21.78	23.15	.8	4792	26.09	27.81
47.0	4538	21.90	23.28	54.0	4799	26.22	27.95
.2	4545	22.02	23.41	.2	4806	26.34	28.05
.4	4553	22.15	23.54	.4	4814	26.47	28.22
.6	4560	22.27	23.67	.6	4821	26.59	28.36
.8	4568	22.39	23.80	.8	4829	26.72	28.49

TABLE 83.[1]—REFRACTIVE INDEXES AND ZEISS IMMERSION REFRACTOMETER READINGS[2] OF ALCOHOL-WATER MIXTURES AT 20 AND 25°C.—(*Continued*)

Scale reading	Refractive index	Per cent alcohol by volume		Scale reading	Refractive index	Per cent alcohol by volume	
		20°C.	25°C.			20°C.	25°C.
55.0	1.34836	26.85	28.63	62.0	1.35095	31.29	33.60
.2	4844	26.97	28.77	.2	5102	31.43	33.75
.4	4851	27.10	28.90	.4	5110	31.56	33.90
.6	4858	27.23	29.04	.6	5117	31.69	34.05
.8	4866	27.35	29.18	.8	5124	31.83	34.21
56.0	4873	27.48	29.31	63.0	5132	31.96	34.36
.2	4880	27.60	29.45	.2	5139	32.10	34.52
.4	4888	27.73	29.58	.4	5146	32.23	34.67
.6	4895	27.85	29.72	.6	5154	32.37	34.83
.8	4903	27.98	29.86	.8	5161	32.51	34.98
57.0	4910	28.10	29.99	64.0	5168	32.65	35.15
.2	4918	28.23	30.13	.2	5176	32.79	35.31
.4	4925	28.35	30.27	.4	5183	32.92	35.48
.6	4932	28.48	30.41	.6	5190	33.06	35.64
.8	4940	28.60	30.55	.8	5198	33.20	35.80
58.0	4947	28.73	30.69	65.0	5205	33.34	35.97
.2	4954	28.86	30.83	.2	5212	33.48	36.13
.4	4962	28.98	30.97	.4	5220	33.62	36.30
.6	4969	29.11	31.11	.6	5227	33.76	36.46
.8	4977	29.23	31.25	.8	5234	33.90	36.63
59.0	4984	29.36	31.40	66.0	5242	34.04	36.79
.2	4991	29.49	31.54	.2	5249	34.18	36.96
.4	4999	29.61	31.68	.4	5256	34.33	37.13
.6	5006	29.74	31.83	.6	5264	34.47	37.30
.8	5014	29.87	31.97	.8	5271	34.62	37.48
60.0	5021	29.99	32.12	67.0	5278	34.76	37.65
.2	5028	30.12	32.27	.2	5286	34.91	37.83
.4	5036	30.25	32.41	.4	5293	35.05	38.00
.6	5043	30.38	32.56	.6	5300	35.20	38.18
.8	5050	30.51	32.71	.8	5308	35.35	38.35
61.0	5058	30.64	32.86	68.0	5315	35.50	38.53
.2	5065	30.77	33.01	.2	5322	35.65	38.70
.4	5073	30.90	33.16	.4	5329	35.80	38.88
.6	5080	31.03	33.31	.6	5337	35.95	39.06
.8	5087	31.16	33.46	.8	5344	36.10	39.24

TABLE 83.[1]—REFRACTIVE INDEXES AND ZEISS IMMERSION REFRACTOMETER READINGS[2] OF ALCOHOL-WATER MIXTURES AT 20 AND 25°C.—(*Concluded*)

Scale reading	Refractive index	Per cent alcohol by volume		Scale reading	Refractive index	Per cent alcohol by volume	
		20°C.	25°C.			20°C.	25°C.
69.0	1.35351	36.25	39.43	75.0	1.35570	41.23	45.07
.2	5359	36.41	39.61	.2	5577	41.41	45.29
.4	5366	36.56	39.80	.4	5584	41.58	45.50
.6	5373	36.72	39.98	.6	5592	41.76	45.71
.8	5381	36.87	40.17	.8	5599	41.94	45.92
70.0	5388	37.02	40.35	76.0	5606	42.12	46.12
.2	5395	37.19	40.53	.2	5613	42.30	46.34
.4	5402	37.35	40.72	.4	5621	42.48	46.56
.6	5410	37.51	40.90	.6	5628	42.66	46.78
.8	5417	37.67	41.08	.8	5635	42.84	47.00
71.0	5424	37.83	41.27	77.0	5642	43.02	47.23
.2	5432	37.99	41.46	.2	5650	43.20	47.45
.4	5439	38.16	41.64	.4	5657	43.39	47.68
.6	5446	38.32	41.83	.6	5664	43.57	47.91
.8	5454	38.49	42.02	.8	5671	43.76	48.14
72.0	5461	38.65	42.21	78.0	5678	43.94	48.37
.2	5468	38.82	42.40	.2	5686	44.13	48.60
.4	5475	38.98	42.58	.4	5693	44.32	48.84
.6	5483	39.16	42.77	.6	5700	44.51	49.07
.8	5490	39.33	42.96	.8	5707	44.70	49.31
73.0	5497	39.50	43.15	79.0	5715	44.89	49.54
.2	5504	39.67	43.33	.2	5722	45.08	49.77
.4	5512	39.84	43.52	.4	5729	45.28	50.01
.6	5519	40.02	43.70	.6	5736	45.48	50.24
.8	5526	40.19	43.89	.8	5744	45.68	50.48
74.0	5533	40.36	44.08				
.2	5541	40.53	44.28				
.4	5548	40.71	44.48				
.6	5555	40.88	44.67				
.8	5563	41.05	44.87				

[1] Compiled from St. John's table, based on the data of DOROSCHEVSKII and DVORZHAN-CHIK: *J. Russ. Phys.-Chem. Soc.*, **1908,** 101.

[2] Scale readings refer only to instruments calibrated on the Pulfrich Scale, see p. 17. If the refractometer used is calibrated in certain other units, the conversion table, page 18, must be used.

1. U. S. Pharmacopœia Test.[1]—A very simple test, which has been widely employed, is that based on oxidation of the alcohol by potassium permanganate and detection of the formaldehyde by acidified fuchsin-sulphurous acid.

Reagents. a. Potassium Permanganate.—Dissolve 3 grams of potassium permanganate in 100 cc. of distilled water containing 15 cc. of phosphoric acid. By using water previously distilled from potassium permanganate solution the keeping qualities of the reagent are improved.

b. Oxalic Acid Solution.—Dissolve 5 grams of oxalic acid in a solution made by diluting 50 cc. of sulphuric acid (sp. gr. 1.84) to 100 cc.

c. Fuchsin-sulphurous Acid.—Dissolve 0.2 gram of rosaniline, or an equivalent weight of its salt, in 120 cc. of hot water, cool, and add to this a solution of 2 grams of sodium bisulphite in 20 cc. of water. Finally add 2 cc. of concentrated hydrochloric acid and dilute the whole to 200 cc. This solution should become colorless, or nearly so, after standing. It keeps well if protected from the air. The fuchsin-aldehyde reagent described on page 566 can be substituted for the rosaniline solution if desired, but in either case, to obtain a nearly colorless reagent, only the purest product obtainable should be used.

Procedure.—Dilute the alcoholic distillate with water to contain 5 per cent by volume of alcohol. To 2 cc. of this diluted alcohol, in a test tube, add 1 cc. of the potassium permanganate solution. Allow to stand for 10 minutes; decolorize by adding 1 cc. of the oxalic acid solution, shake, and let stand for 1 minute until colorless. Add 2 cc. of fuchsin-sulphurous acid and mix thoroughly. Let stand for 20 minutes for the full color to develop. Methyl alcohol gives a violet color which is permanent for several hours at least. With traces the color may not develop for 1 hour.

Notes.—The potassium permanganate oxidizes a portion of the alcohol to aldehyde, the methyl alcohol to formaldehyde, and the ethyl alcohol to acetaldehyde. The fuchsin-sulphurous acid is not a specific test for formaldehyde but is a test for aldehydes in general. In this case, the inhibition of the reaction for

[1] DENIGÈS: *Compt. rend.*, **1910, 832**; "U. S. Pharmacopœia," 10th Decennial Revision, p. 39; CHAPIN: *Ind. Eng. Chem.*, **1921**, 543; WRIGHT: *ibid.*, **1927**, 750; LEVIN, UHRIG and STEHR: *Ind. Eng. Chem., Anal. Ed.*, **1939**, 135.

acetaldehyde is accomplished by the acidulation with sulphuric acid.

The test as originally described in the ninth U. S. Pharmacopœia was severely criticized because a positive test was given with pure ethyl alcohol due to the formation of traces of formaldehyde by the oxidizing agent. This has been found to be due to too high a temperature during oxidation and when the fuchsin-sulphurous acid is added. Especial care should be taken that the temperature does not exceed 25°C., the test tube being placed in cold water if necessary.

The test as described is readily sensitive to 1 part of methyl alcohol in 500 of ethyl alcohol and can be made more delicate by fractionation of the distillate, the test being applied to the 1-cc. fraction coming over first from a 10-cc. portion. Increased delicacy, however, is without practical importance.

Numerous substances are formed in some amount during alcoholic fermentation, which will give colors similar to, although usually much less intense, than that given by the formaldehyde. Of these perhaps the most troublesome in food materials would be glycerol, invariably present in fermented products, and pectin, present in fruits as the unstable methyl ester of pectic acid, and hence found in apple and similar fruit juices. For this reason a positive test should never be taken as conclusive unless it has been obtained on a distillate of the original material. An exhaustive list of interfering substances is given by Georgia and Morales.[1]

For the lower ranges of colors it is possible, by carefully standardizing the process, especially as regards time and temperature of the reactions, to determine approximately the proportion of methyl alcohol present. Many valuable suggestions of practical importance will be found especially in the papers of Wright and of Georgia and Morales cited. The use of a spectrophotometer has been found helpful by Geyer.[2]

2. Resorcin Test.[3]—Place 10 cc. of the liquid to be tested, having an alcohol concentration of about 10 per cent, in a test tube surrounded by cold water. Wind a length of medium-sized copper wire around a pencil so that the closely coiled

[1] *Ind. Eng. Chem.*, **1926**, 304.

[2] *J. Assoc. Off. Agr. Chem.*, **1939**, 151.

[3] MULLIKEN and SCUDDER: *Am. Chem. J.*, **1899**, 266; **1900**, 444.

spiral will form a cylinder about 1 in. long, leaving about 8 in. unwound for a handle. Heat the spiral red hot in the oxidizing flame of a burner, plunge it to the bottom of the test tube, and hold it there for 1 to 2 seconds. Repeat this five or six times. Filter the solution and boil it gently until any odor of acetaldehyde is no longer apparent. Cool the solution, add 1 drop of a 1:200 aqueous solution of resorcin, and pour it cautiously on top of 5 to 10 cc. of concentrated sulphuric acid in a test tube. Allow to stand for 3 minutes, then shake *very gently* for 1 minute, causing a very gradual mixing of a portion of the two layers. With 2 per cent of methyl alcohol a rose-red ring will appear at the junction of the liquids, and, if the quantity is somewhat greater than this, characteristic rose-red flocks will separate when the tube is gently shaken.

Notes.—The treatment with the hot spiral oxidizes a part of the alcohol to aldehyde, the methyl alcohol to formaldehyde, and the ethyl alcohol to acetaldehyde. By the boiling the acetaldehyde (and some of the formaldehyde) is expelled, thus increasing the delicacy of the test, since acetaldehyde gives with resorcin a yellowish-brown ring and flocks, which tend to obscure the formaldehyde reaction.

Other methods for the detection of formaldehyde, such as are described on pages 102 and 103, might be employed, but care should be taken not to use too delicate a test, since traces of formaldehyde are produced by the oxidation of ethyl alcohol. For this reason the gallic acid test (page 103), an extremely characteristic test for formaldehyde and one not readily obscured by the presence of other compounds, is not so well suited. There is a distinct advantage, on the other hand, in employing several tests, based on differing reactions, as the resorcin and the phenylhydrazine test (footnote, p. 144), since foreign substances that might interfere with one reagent might be less likely to do so with another.

The test as described, even including the production of the more characteristic flocks, is probably delicate enough to detect methyl alcohol in the quantities in which it would be likely to be added to a food product for commercial profit, but, if a negative result is obtained and there is still reason to believe that methyl alcohol may be present, a portion of the alcoholic liquid may be fractionated several times through a Glinsky, Hempel, or

other suitable apparatus, and the test repeated on the concentrated solution.

The test may also be made more delicate by conducting the oxidation with a solution of chromic acid[1] instead of solid copper oxide in wire form. This is accomplished according to the directions of Gettler as follows: Neutralize 50 cc. of the sample with sodium carbonate solution and phenolphthalein, and slowly distill 25 cc. To the distillate add 100 cc. of 10 per cent sulphuric acid, 6 grams of potassium bichromate, and let the mixture stand 10 minutes. Very slowly distill 30 cc., using a 250-cc. distilling flask. The distillation of the 30 cc. should require 1 hour. The distillate contains most of the acetaldehyde but very little of the formaldehyde, and is to be rejected. Then continue the distillation a little faster, until 60 cc. are obtained. This part of the distillate contains most of the formaldehyde and hardly any of the acetaldehyde, and is to be used for the tests.

The methods for the detection of methyl alcohol, adopted as provisional by the Association of Official Agricultural Chemists,[2] are those of Trillat[3] and of Riche and Bardy.[4] These are, however, long and tedious and suited only to experienced chemists, and hence are omitted here in favor of the shorter and simpler methods described. Critical summaries of the more important tests for detecting methyl alcohol have been published by Scudder[5] and by Gettler.[6]

3. Refractometer Method.—The densities of pure methyl alcohol and of ethyl alcohol are not far apart, the former being 0.7965 and the latter 0.7939 at $\frac{15°}{15°}$C. The refractive indexes, however, are quite different, being 1.3614 at 20°C. for ethyl alcohol and 1.3281 at the same temperature for methyl alcohol, a difference easily measured. It has been proposed to utilize this distinction for the detection of methyl alcohol in ethyl

[1] VORISEK: *J. Soc. Chem. Ind.*, **1909**, 823; BACON: U. S. Dept. Agr., *Bur. of Chemistry, Circ.* **74**; GETTLER: *J. Biol. Chem.*, **42, 1920**, 311.

[2] "Official Methods of Analysis," **1935**, p. 173.

[3] *Compt. rend.*, **1898**, 232; *Analyst*, **1899**, 211.

[4] *Compt. rend.*, **1875**, 1076; LEACH-WINTON: "Food Inspection and Analysis," 4th ed., p. 783.

[5] *J. Am. Chem. Soc.*, **1905**, 892.

[6] *Loc. cit.*

alcohol, the refraction being determined by means of the immersion refractometer.

To do this, use the distillate obtained in determining the per cent of alcohol from the specific gravity (page 486) and determine its reading on the immersion refractometer at 20°C. if this has not already been done. The following table (page 514) worked out by Leach and Lythgoe,[1] shows the scale reading on the refractometer for each per cent of methyl and ethyl alcohol present as determined from the density. If the refractometer reading is appreciably lower than corresponds to the percent of ethyl alcohol shown by the density, the presence of methyl alcohol is indicated.

Moreover, the addition of methyl to ethyl alcohol lowers the refraction in direct proportion to the amount added. Hence, the quantitative determination may be made by interpolation in Table 84 using the figures for pure ethyl and methyl alcohol of the same alcoholic strength as the distillate.

Example.—Suppose the distillate in an alcohol determination has a density at $\frac{20°}{4°}$C. of 0.97076, corresponding to 18.40 per cent of ethyl alcohol by weight, and has a refraction of 35.8 on the immersion refractometer at 20°C. By interpolation in Table 84 the readings of ethyl and methyl alcohol, corresponding to 18.40 per cent are 47.3 and 25.4, respectively, the difference being 21.9.

$$47.3 - 35.8 = 11.5; \frac{11.5}{21.9} \times 100 = 52.5.$$

Hence 52.5 per cent of the alcohol present is methyl.

Note.—The difference in refraction for the two alcohols, as will be seen from the table, varies considerably for different strengths. In the case of methyl alcohol the refraction increases with increasing concentration until it reaches a maximum at about 50 per cent by weight. In the case of ethyl alcohol the maximum refraction is reached at 75 per cent, but the decrease above this concentration is by no means as rapid as with methyl alcohol. This means that the delicacy of the method is consider-

[1] LEACH and LYTHGOE: *J. Am. Chem. Soc.*, **1905**, 964. Later tables, of slightly greater accuracy, have been published by Wagner, *Z. anal. Chem.*, **46**, 508, and by Doroshevskii and Dvorzhanchik, *J. Russ. Phys.-Chem. Soc.*, **41**, 951.

ably greater for the higher concentrations of alcohol. In the case of most food products, however, the necessity for distilling without loss of alcohol restricts the method to the lower concentrations of alcohol.

The determination must be made with great care, especially as regards temperature control, both in determining the refraction and the density, and several settings of the refractometer should be made in order to minimize errors of parallax. Indications of less than 0.5 per cent of methyl alcohol should be disregarded unless confirmed by some more distinctive qualitative test, in fact this procedure would be safest in any case. The method, even with these limitations, is rapid and convenient, and for reasonable proportions of methyl alcohol perhaps the best.

Although the method is given here in the original form, and as almost universally employed in this country for the estimation of methyl alcohol, Williams[1] has pointed out that it was intended to be used only with the so-called "Hehner alcohol table," based on determinations of specific gravity at $\dfrac{15.6°C.}{15.6°C.}$, and does not give correct values when used with the $\dfrac{20°}{4°}$ table, the result for methyl alcohol being, in some instances even a negative value. Williams suggests a similar method, based on more recent tables and applicable to alcohol itself and to liquids relatively high in alcohol. The method is practically as described above, except that the alcoholic distillate, or the original alcoholic sample itself where suitable, is diluted after determining its specific gravity at $\dfrac{20°}{4°}$ so as to contain exactly 20 per cent of total alcohol by volume, calculated as ethyl alcohol. For example, if the sample or distillate were found to contain 64.7 per cent alcohol by volume, then 30.9 cc. (20.0 ÷ 64.7 × 100 = 30.9) made up to 100 cc. with water, at 20°C., would be the desired concentration. On this solution the immersion refractometer reading R at 20°C. is obtained, and from the specific gravity determined with a pyknometer at $\dfrac{20°}{4°}$C. the exact percentage of alcohol[2] by volume P

[1] *Ind. Eng. Chem.*, **1927**, 844.

[2] This figure may be somewhat above 20.0, depending upon the amount of methyl alcohol present. It will of course be exactly 20.0 per cent if ethyl alcohol only is present, but higher in the presence of methyl alcohol as well, reaching 21.5 per cent for pure methyl alcohol.

is taken from Table 82. From the difference in these two values $(R - P)$ the approximate percentage by volume of methyl alcohol in the total alcohol can be found by the expression

$$\frac{23.8 - (R - P)}{0.2} = \text{per cent.}$$

TABLE 84.—SCALE READINGS ON ZEISS IMMERSION REFRACTOMETER* AT 20°C., CORRESPONDING TO EACH PER CENT BY WEIGHT OF METHYL AND ETHYL ALCOHOLS

| Per cent. alcohol by weight | Scale readings | | Per cent. alcohol by weight | Scale readings | | Per cent. alcohol by weight | Scale readings | | Per cent. alcohol by weight | Scale readings | |
	Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol
0	14.5	14.5	26	30.3	61.9	51	39.7	91.1	76	29.0	101.0
1	14.8	16.0	27	30.9	63.7	52	39.6	91.8	77	28.3	100.9
2	15.4	17.6	28	31.6	65.5	53	39.6	92.4	78	27.6	100.9
3	16.0	19.1	29	32.2	67.2	54	39.5	93.0	79	26.8	100.8
4	16.6	20.7	30	32.8	69.0	55	39.4	93.6	80	26.0	100.7
5	17.2	22.3	31	33.5	70.4	56	39.2	94.1	81	25.1	100.6
6	17.8	24.1	32	34.1	71.7	57	39.0	94.7	82	24.3	100.5
7	18.4	25.9	33	34.7	73.1	58	38.6	95.2	83	23.6	100.4
8	19.0	27.8	34	35.2	74.4	59	38.3	95.7	84	22.8	100.3
9	19.6	29.6	35	35.8	75.8	60	37.9	96.2	85	21.8	100.1
10	20.2	31.4	36	36.3	76.9	61	37.5	96.7	86	20.8	99.8
11	20.8	33.2	37	36.8	78.0	62	37.0	97.1	87	19.7	99.5
12	21.4	35.0	38	37.3	79.1	63	36.5	97.5	88	18.6	99.2
13	22.0	36.9	39	37.7	80.2	64	36.0	98.0	89	17.3	98.9
14	22.6	38.7	40	38.1	81.3	65	35.5	98.3	90	16.1	98.6
15	23.2	40.5	41	38.4	82.3	66	35.0	98.7	91	14.9	98.3
16	23.9	42.5	42	38.8	83.3	67	34.5	99.1	92	13.7	97.8
17	24.5	44.5	43	39.2	84.2	68	34.0	99.4	93	12.4	97.2
18	25.2	46.5	44	39.3	85.2	69	33.5	99.7	94	11.0	96.4
19	25.8	48.5	45	39.4	86.2	70	33.0	100.0	95	9.6	95.7
20	26.5	50.5	46	39.5	87.0	71	32.3	100.2	96	8.2	94.9
21	27.1	52.4	47	39.6	87.8	72	31.7	100.4	97	6.7	94.0
22	27.8	54.3	48	39.7	88.7	73	31.1	100.6	98	3.5	93.0
23	28.4	56.3	49	39.8	89.5	74	30.4	100.8	99	3.5	92.0
24	29.1	58.2	50	39.8	90.3	75	29.7	101.0	100	2.0	91.0
25	29.7	60.1									

* Scale readings are applicable only to instruments calibrated on the Pulfrich scale (see p. 17). If the instrument used is calibrated in certain other arbitrary units the refractive index corresponding to the observed reading may be converted into the proper Zeiss reading by means of the table on page 18.

Isopropyl Alcohol.—Isopropyl alcohol, although not common in beverages, has been found in extracts and flavors as a sub-

stitute for ethyl alcohol and in drugs as an organic solvent. The methods for its detection and determination are based in general upon its oxidation to acetone. A typical procedure consists of oxidation with potassium bichromate, distillation of the acetone formed and its determination by the method of the U. S. Pharmacopœia.[1] A qualitative test is made for acetone in the original sample, and if found it is removed with paraformaldehyde.[2] The procedure is as follows:[3]

Qualitative Tests.—*a.* Distill a portion of the sample and collect the first 5 cc. To 2 cc. of the distillate add 5 cc. of a 5 per cent alcoholic solution of o-nitrobenzaldehyde and 1 cc. of 10 per cent sodium hydroxide. Mix and shake with a little chloroform. A blue color in the chloroform shows the presence of acetone.

b.[4] To 2 cc. of the distillate in a small test tube add an equal volume of a freshly prepared solution containing 20 grams of sodium nitroprusside and 20 grams of ammonium chloride per 100 cc. and mix thoroughly. Carefully overlay this mixture with 3 to 4 cc. of concentrated ammonium hydroxide. A deep purple coloration appearing at the interface between the two liquid layers within 10 minutes shows the presence of acetone.

Determination. *a. If Acetone is Present.*—Place 1.5 grams of paraformaldehyde in a 200-cc. volumetric flask and add about 20 cc. of normal sodium hydroxide solution. Pipette an aliquot containing not over 0.8 gram of alcohol into the flask. (An approximation to the alcoholic content may be had by a specific-gravity determination and reference to the ethyl alcohol tables.) Dilute the mixture with water to about 100 cc. Connect the flask to a reflux condenser and heat slowly on a hot plate just to boiling. Remove the hot plate, wash down the condenser, and when cool make to the mark and mix. Filter through a dry filter, if necessary pouring the solution back through the filter until a clear filtrate is obtained. Pipette 100 cc. into a 500-cc. Erlenmeyer flask, add 5 grams of potassium bichromate, and when most of the salt has dissolved, add 100 cc. of sulphuric acid

[1] MESSINGER: *Ber.*, **1888**, 3336; GOODWIN: *J. Am. Chem. Soc.*, **1920**, 39; *U. S. Pharmacopœia*, Eleventh Decennial Revision.

[2] HOFF and MACOUN: *Analyst*, **1933**, 749.

[3] STANLEY: *J. Assoc. Off. Agr. Chem.*, **1939**, 594.

[4] RAE: *Pharm. J.*, **1926**, 630.

$(1 + 3)$. Stopper the flask, swirl, and let it stand 30 minutes. Add 100 cc. of 25 per cent ferrous sulphate solution. Connect the flask to a vertical condenser through a foam trap. Slowly distill about 100 cc. into a 500-cc. volumetric flask containing 200 to 300 cc. of cold water. Dilute to the mark, mix, and pipette 25 cc. into a glass-stoppered flask containing 25 cc. of normal sodium hydroxide; add 50 cc. of standard $0.1N$ iodine while swirling the flask. Allow it to stand 15 minutes. Add 26 cc. of normal hydrochloric acid and at once titrate the residual iodine with standard $0.1N$ sodium thiosulphate solution, adding starch solution when the iodine color is nearly discharged. Each cubic centimeter of $0.1N$ iodine consumed in the reaction corresponds to 0.001001 gram of isopropyl alcohol ($CH_3CHOHCH_3$).

b. If Acetone is not Present.—Into a 500-cc. Erlenmeyer flask containing 50 cc. of approximately $2N$ potassium bichromate, pipette an aliquot containing not over 0.8 gram of alcohol. Dilute to about 100 cc. with water and continue as directed previously, beginning with "Add 100 cc. of sulphuric acid $(1 + 3)$. . . . ".

Notes.—All stoppers used in the apparatus should be covered with tin foil. Formaldehyde reacts with acetone in alkaline solution to form a non-volatile resin.

If the isopropyl alcohol were mixed with water only it could be determined from the density or refractive index as with ethyl or methyl alcohols. Isopropyl alcohol has a density $\left(\dfrac{20°}{4°}\right)$ of 0.7846; boiling point, 82.2°C.; refractive index at 20°, 1.3744. Tables for the specific gravity and refractive index of such mixtures are available.[1]

The alcohol may be accompanied, however, by other volatile, water-soluble alcohols, aldehydes, and ketones, so that these methods are usually out of the question, and the best procedure is by oxidation to acetone, the aldehydes and primary alcohols being oxidized to the corresponding acids.

The condensation of acetone with *o*-nitrobenzaldehyde to form indigo, used as a qualitative test above, has also been made the basis of a quantitative method by comparison with suitable standards in aqueous solution.[2]

[1] BATSCHA and REZNEK: *J. Assoc. Off. Agr. Chem.*, **1937**, 107.
[2] ADAMS and NICHOLLS: *Analyst*, **1929**, 2.

WINE

The term *wine*, without further qualification, is universally understood to mean the product of alcoholic fermentation of the juice of the grape. This not only excludes the fermented product of other fruit juices, but when the definition is made more rigid, as in a legal standard, it is usually specified that the wine shall be made by the "usual cellar treatment" (see page 540). The purpose of this is not only to restrict the original crude material to the grape juice, but also to limit all additions to those substances that occur naturally in sound grapes, or which experience has shown to be of distinct benefit to the flavor or keeping qualities of the wine.

Classification.—On account of the exceedingly great variation in the character of the grapes grown in different soils and in localities widely separated, to say nothing of the changes in appearance, flavor, and composition brought about by differences in the methods of manufacture, wines are placed on the market in almost infinite variety. Many of these are distinguished and well known in commerce by the name of a region, a locality, or a particular vineyard; the wines of a certain vineyard may be further differentiated according to the year of the vintage; finally, the special brands and names that appear may be numbered among the thousands. With such trade distinctions, however, it is not necessary to deal for the purposes of analysis. Based on essential differences in chemical composition, natural wines may be grouped into three divisions, each including two subdivisions, red and white, depending on whether the color is extracted from the skins of the grapes or not:

    a. Dry wines.

    b. Sweet wines.

    c. Sparkling wines.

A *dry wine* is one in which practically all the sugar, certainly all that can be perceived by the taste, has been changed by fermentation. A *sweet wine* is one in which sufficient sugar remains in the finished product to give it a noticeable sweet taste. This difference in the sugar content of the wine is intentional and is due to the desire of the manufacturer in one case to carry on the fermentation continuously until all the sugar has been converted to the maximum amount of alcohol and carbon dioxide,

while in the latter case the progress of the fermentation is stopped by the addition of alcohol while part of the sugar still remains unchanged. Dry wines seldom are absolutely free from sugar, the amount ranging from a few hundredths up to several tenths of 1 per cent. Sweet wine contains, of course, much more, ranging from 2 or 3 up to 10 per cent or more. The Federal standards (see page 540) have set as an arbitrary line between dry and sweet wines the presence of 1 gram of sugars per 100 cc.

A *sparkling wine* is one which is charged with an excess of carbon dioxide, usually enough to cause a pressure of several atmospheres in the bottle at ordinary temperature. With natural wines this condition is brought about by a supplementary fermentation in the bottle; with artificial wines it may be due to carbonating, in a manner similar to the production of effervescent drinks. The opposite term to sparkling wine is *still wine*, but this is naturally included in the two classes just considered.

Another classification, which is at times of value in considering the analytical characteristics of wine, is into *natural* and *fortified* wines. The former are those to which neither sugar nor alcohol has been added but in which the fermentation has been allowed to proceed until checked naturally by lack of sugar or increase of alcoholic strength. The dry wines are typical of this class. The fortified wines have had alcohol, either in the form of brandy (wine distillate) or from some other source, added. This addition ordinarily takes place before the fermentation is finished, so that a certain proportion of the sugar is left unfermented and the wine is a sweet wine. Wines of this type may contain 20 per cent or more of alcohol; whereas the natural fermentation does not yield over 14.5.

Manufacture.—Only the merest outline of the methods used in producing wine, enough to give a general survey of the chemical changes brought about, can be given here.

a. Pressing.—The grapes are picked, usually at the time when they contain the most sugar, and carted to the winery for pressing. If the grapes are to be used for the production of white wine they are crushed and pressed so that only the juice shall be fermented; if for red wine, the entire pulp after crushing is placed in the fermenting vats in order to extract the color during fermentation. The crushing and pressing are done by rollers and screw

or hydraulic presses, the juice that is pressed out constituting the *must* and the residue left in the press the *pomace* or *marc*.

b. Fermenting.—The alcoholic fermentation of the must is due to the presence of various yeasts, of which the most important is the so-called "true wine yeast," *Saccharomyces ellipsoideus*. The *zymase*, or enzyme, present in the yeast cells, acts on the dissolved sugar, changing it to alcohol and carbon dioxide. These yeasts are present on the skins of the grapes, so that under ordinarily favorable conditions the must will begin to ferment almost immediately, as shown by the increase in temperature, formation of gas bubbles, and change from a sweet taste to an acid and alcoholic one. On account, however, of the presence of other less desirable yeasts the winemaker often adds to the must pure cultures of special yeasts, thereby securing better control of the fermentation and a cleaner product.

The fermentation is carried on in casks or vats at a temperature varying between 55 and 85°F., the temperature and details of the process differing somewhat for white and for red wines. After fermenting, the wine is drawn off into other casks, frequently sulphured to prevent the further development of microorganisms, and allowed to ripen for several months or until the following autumn, being occasionally drawn or "racked" off into other containers, in order to remove the sediment and by oxidation hasten the precipitation of undesirable albuminous substances and improve the flavor. "Finings," usually gelatin or egg albumen, may be added in small quantity to assist in this precipitation and clarification.

c. Bottling.—When the wine has ceased forming a deposit, is bright and clear, and has reached its optimum quality, it is bottled, the object being to prevent further deleterious change by protecting it from oxidation or the action of microorganisms. After bottling, the wine improves for several months and then remains with little change except for a very gradual development of the highest qualities of flavor for many years, if kept under suitable conditions.

Sparkling Wine.—The preliminary fermentation of sparkling wines is carried out in practically the manner described above. After blending, sufficient sugar solution is added to develop a pressure of about 5 atmospheres during the subsequent bottle fermentation, special yeast is added, and the wine bottled and

corked. At the end of the bottle fermentation, which lasts from 6 months to 10 years, the bottles are gradually inverted in the racks, thus collecting the sediment near the cork. By dexterously uncorking the bottle momentarily, this sediment is removed. Some more sugar is often added and the bottle again securely corked.

General Composition.—The principal ingredients present in the must are sugar (dextrose); organic acids, principally tartaric, malic, and tannic; albuminoids; dextrin, pectin, and other mucilaginous carbohydrates; and small amounts of various flavoring substances. Of these, the sugar may almost entirely disappear during fermentation, or a considerable proportion may remain in the finished wine. The tartaric acid, which varies considerably in amount between red and white wines, is largely removed by the precipitants used in the fining process. In addition, part of the acids may serve as food for microorganisms during the process of manufacture and a small proportion of acetic acid may be formed by subsidiary fermentations. The albuminoids are largely removed, partly as nourishment for the yeast and partly by precipitation during the fermentation and cellar treatment. The mucilaginous substances are largely precipitated as the fermentation proceeds on account of their relative insolubility in dilute alcohol.

The finished wine, then, while retaining in part the character of the must, will have the proportions of the constituents largely altered and contain some new ones. During the aging of the wine there is a notable increase in volatile acids and esters and decreases in extract, total tartaric acid, potassium acid tartrate, color, and tannin. This is largely due to oxidation and the precipitation of wine "lees," composed of potassium acid tartrate and oxidized color and tannin. From a chemical standpoint its important constituents are: Alcohol, glycerol, invert sugar, acids, both fixed and volatile, tannin, and color.

Forms of Adulteration.—The most common methods of adulterating wine consist either in some improper treatment during the process of manufacture, the addition of some improper substance to the wine itself, or the substitution of an inferior or less desirable product for the genuine article.

With reference, especially, to materials added during the process of manufacture, the central idea of all proper additions is

that they should be such as to improve the product and not to defraud the purchaser. One thing is *amelioration* or improvement of the wine; the other is *adulteration*.

The additions that are commonly allowed in wine are for the purposes of controlling the fermentation or character of the wine, or for correcting natural defects due to climatic or seasonal conditions. Of these the most important are:[1]

The usual agents, such as tannin, albumen, casein, or gelatin, which are used as "fining" agents in the clarification of the wine;

Sulphurous acid or bisulphites in limited quantities (see page 548), for the control of the fermentation and to assist in keeping the wine;

In the case of excessive acidity, neutralizing agents that do not render the wine injurious to health, such as neutral potassium tartrate or calcium carbonate;

In the case of deficient acidity, tartaric acid;

In the case of deficiency in saccharine matter, condensed grape must, or a pure dry sugar.

The addition of grape brandy for the fortification of sweet wine is also permitted in accordance with the Sweet Wine Fortification Act of June 7, 1906.

Additions that would be regarded as constituting adulteration would be adding water to increase the bulk of the product, the addition of ordinary alcohol, or adding fermented liquids derived from other sources than the grape. The addition of brandy, even, must be only to certain classes of wines and then under definite restrictions (see page 541). Such additions as artificial preservatives or color would naturally constitute adulteration under the general provisions of the Food and Drug Act, except in a few instances, when they may be added if properly declared.

The addition of gypsum or plaster of Paris to the wine is also generally regarded as adulteration, and either it is forbidden or the amount that may be used is strictly limited. The object of this practice, commercially known as "plastering," is to improve the wine by precipitating a certain proportion of the potassium acid tartrate as the insoluble calcium salt, thus bringing about a sort of clarification which improves the color and keeping qualities of the wine. The increased amount of potas-

[1] U. S. Dept. Agr., *Food Inspection Decision* **156.**

sium sulphate thus added is, however, considered decidedly objectionable.[1]

The practice is also in vogue of adding to the *marc* or *pomace, i.e.,* the residue of pulp, skins, seeds, etc., from which the must has been drawn off, a solution of sugar in water. This readily ferments and yields a considerable quantity of "wine" which is used for blending or sometimes, by the help of saccharin, coloring matter, and preservatives, is put on the market directly. Such a product must be so labeled as to indicate its true character.

Some forms of adulteration are possibly better described as substitution or misbranding. The use of raisins in the manufacture yields a wine, which, from the nature of the raw material, would be quite difficult to distinguish from a product made by the fermentation of fresh grapes. It is required that such a wine be so labeled as to indicate its origin, but the problem of detecting it is not an easy one so far as chemical tests are concerned, because the constituents of raisins are for the most part the same as those of grapes, and the solution obtained by soaking them in water can be fermented to yield a very similar product to ordinary wine. Such a product, however, can usually be detected by its characteristic flavor, this being readily distinguished by those familiar with the taste of genuine wines.

Misbranding as to variety, *i.e.,* so labeling an inferior product as to indicate that it is made from a standard or more highly prized variety of grape, is hardly to be detected by chemical tests, but must usually be shown by differences in character and flavor which are apparent to the trained palate. The labeling of a domestic wine as being a foreign product can often be detected by analysis, since the wines produced in this country are in many cases distinctly different in their exact composition from the foreign product which they are made to resemble. Such wines must be labeled with the word "type" or with the name of the state or locality where produced.

Methods of Analysis

Statement of Results.—The most convenient form in which to express the analytical results is as grams per 100 cc. of wine,

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[1] According to Neufeld: *Z. Nahr.-Genussm,* **27, 1914,** 299, the presence of sulphates, calculated as potassium sulphate, in excess of 2 grams per liter has been shown to be distinctly harmful physiologically.

and unless some statement is made to the contrary they should be so stated in the determinations that follow. If the *per cent* of any constituent is desired this can be readily calculated from the specific gravity of the sample.

Specific Gravity.—This may be determined at $\frac{20°}{20°}$C. by means of the pyknometer or Westphal balance as described under General Methods, pages 1 to 5.

Alcohol.—Use 100 cc. of the sample, 50 cc. of water, and determine the alcohol as described on page 486, noting the directions in regard to neutralizing the acidity if necessary. If the sample froths badly this may be prevented by the addition of a little tannic acid, which precipitates the proteins. Calculate the result as per cent of alcohol by volume. If it is desired to know also the grams of alcohol per 100 cc., as for calculating alcohol ratios in interpreting the results, this may be found by multiplying the per cent by volume by the specific gravity of absolute alcohol.

A close approximation, usually 0.2 to 0.4 per cent higher, but suitable for many purposes, can be obtained in much less time on the wine directly, without distilling, by the use of the ebullioscope, page 487.

Extract.—On account of the presence of levulose, in considerable proportions, in sweet wines the method to be followed for determinining the total solids or extract varies somewhat with the character of the sample.

Method. a. By Calculation.—Calculate the extract from the specific gravity of the alcohol-free wine by the formula:

$$E = \frac{s - s'}{0.00386},$$

in which s is the specific gravity of the wine, s' the specific gravity of the distillate obtained in the determination of alcohol, and 0.00386 the increase in gravity caused by 1 gram of wine solids in 100 cc. E will be the extract in grams per 100 cc.

If desired, the specific gravity of the alcohol-free wine $(1 + s - s')$ can be referred to Table 36, page 309, for the per cent of extract, which is practically the same method as in the preceding paragraph.

b. By Direct Evaporation.—If the extract, calculated as above, is less than 3 grams per 100 cc. it should be determined directly. Evaporate 50 cc. of the wine on the water bath to a sirupy consistency in a flat-bottomed platinum dish. Dry in the oven at 100°C. for 2½ hours, cool, and weigh.

If the extract, as calculated, is between 3 and 6 grams per 100 cc., evaporate 25 cc. of the wine as directed previously.

If the calculated extract is over 6 grams per 100 cc., the result obtained by method *a* should be accepted and no direct determination made.

Notes.—With sweet wines the drying method is inaccurate on account of the decomposition of levulose at temperatures much above 75°C. (see also page 312).

The calculation by the factor 0.00386 is based on the assumption that this factor, which is the increase in density caused by 1 gram of sucrose in 100 cc. of water, is correct for the solids of wine. This assumption is reasonably correct for sweet wine, in which the extract consists largely of sugars, but is less exact for dry wines, in which the extract consists mainly of non-sugars. In spite of this, the Swiss Association of Analytical Chemists has recommended[1] that the indirect method be employed in all wines on account of the variable results obtained in the direct drying, these varying much with the dish and oven employed.

The extract may also be determined by evaporating a measured quantity of the wine on the water bath to one-fourth its volume, diluting to the original volume, and determining its specific gravity or refractive index. The result may then be taken from the appropriate table, pages 309 and 314. On account, however, of changes in solubility or incipient decomposition during the heating, the results obtained in this way are somewhat less accurate.

Ash.—Use the residue obtained in the determination of extract or, if this was not determined by direct drying, evaporate to dryness 50 cc. of the wine. In either case cautiously char the residue but do not ignite strongly, because of the danger of loss of alkali chlorides and of fusing potassium carbonate. Boil the charred mass several times with small portions of water, decanting each time through an ashless filter, place the filter in the dish and ignite until the ash is white. Add the filtrate to the dish,

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[1] *Mitt. d. Schweizer Gesundh.-Amtes,* **1911,** 447.

evaporate to dryness on the water bath, moisten with ammonium carbonate solution, ignite at a low red heat, cool, and weigh (see also page 24).

Note.—On account of the presence of potassium salts in considerable amounts, the charring of the ash must be done with great care. The addition of ammonium carbonate is to restore the carbon dioxide to carbonates that have been decomposed by the ignition.

Acidity. Total Free Acids. *Method a.*—Neutralize about 250 cc. of recently boiled water with 0.1N sodium hydroxide, using a large porcelain dish and about 2 cc. of phenolphthalein indicator solution. Heat the portion of wine to be titrated to incipient boiling, transfer it to the dish with a portion of the neutralized water, and titrate the whole rapidly to a distinct pink. The quantity of wine to be measured for the test depends on the depth of color of the wine. It may be from 5 cc. for a deeply colored red wine to 25 cc. for a white wine.

Calculate the result as tartaric acid, $H_2C_4H_4O_6$.

Method b.—Measure 25 cc. of the wine into a 250-cc. beaker, heat rapidly to incipient boiling, and immediately titrate with 0.1N sodium hydroxide. Determine the end point with neutral 0.05 per cent azolitmin solution as an outside indicator. Place the indicator in the cavities of a spot plate and spot the wine into the azolitmin solution. The end point is reached when the addition of a few drops of alkali fails to change the color of the indicator.

In tests of this kind it will be found a saving of time to make a preliminary titration to ascertain the acidity approximately before proceeding with the final titrations. Report the result as tartaric acid as in the previous method.

Notes.—The wine is heated before titrating, partly to expel carbon dioxide and partly to decrease the amphoteric effect of the phosphates on the indicator.

A dry mixture of 1 part of phenolphthalein to 100 parts of coarsely powdered potassium sulphate can be placed in the cavities of the spot plate and used as an outside indicator.

The results are calculated as tartaric acid largely through force of custom, this being the principal acid present, though not necessarily as the free acid itself. Lactic acid is also present in distinct amounts. Other ways of stating the result are as grams

of sulphuric acid per liter or as cubic centimeters of normal acid in 100 cc. of wine.

Volatile Acids.—The determination of volatile acids is an important one in drawing conclusions as to the age and soundness of the wine, and the various procedures have been carefully studied. There is no entirely satisfactory method, the ones described being those that have become sanctioned by time and common use. A distinct source of error might be the inclusion of the sulphur dioxide in the volatile acid as commonly determined. Certain wines contain a fairly high proportion of sulphur dioxide used to prevent fermentation in the bottle. With these the sulphur dioxide, calculated as acetic acid, may be as much as the actual volatile acids present. The sulphur dioxide may be determined separately, as under Preservatives, page 112, and allowance made for it, although this is not commonly done.

a.[1]—Measure 50 cc. of wine into a 250-cc. round-bottomed flask and distill by means of a current of steam introduced by a tube reaching to the bottom of the flask, heat also being applied by a small flame in order to reduce the volume of the liquid, and keep it constant at about 25 cc. If much foaming is experienced a little tannin may be added. Distill 200 cc. and titrate with $0.1N$ sodium hydroxide and phenolphthalein. Calculate the acidity as acetic acid, $HC_2H_3O_2$.

Note.—This is the oldest established method for determining volatile acids in wine and is perhaps still the most widely used. The results, however, show considerable differences at times, owing to variations in the apparatus employed and to the direct heating of the sample during the steam distillation. Further, it has been shown by numerous observers[2] that not all the volatile acids are distilled in 200 cc. of distillate except under rigidly prescribed conditions. On the other hand, if larger volumes of distillate are collected, the process becomes tedious and there is danger of distilling notable quantities of lactic acid through entrainment.[3]

[1] Lindemann: *Z. anal. Chem.*, **1883**, 166; U. S. Dept. Agr., *Bur. Chem. Bull.* **107**, p. 86.

[2] Among others see Windisch and Roettgen: *Z. Nahr.-Genussm.*, **1905**, 70; **1911**, 155; Hortvet: *Ind. Eng. Chem.*, **1909**, 31.

[3] Kulisch: *Z. Nahr.-Genussm.*, **1907**, 663.

Many of these difficulties are avoided and the time shortened by having the distilling flask surrounded by boiling water as in the method described below.

b. Hortvet-Sellier Method.[1]—The apparatus, which may be secured from dealers in chemical supplies under the name of the Sellier distilling apparatus, is shown in Fig. 80.[2]

Procedure.—Place 10 cc. of the wine, previously freed from carbon dioxide by heating to incipient boiling, in the inner tube
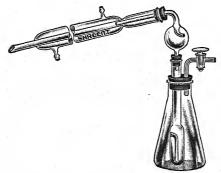
Fig. 80.—Apparatus for volatile acid in wine. (*Courtesy of E. H. Sargent Co.*)

of the apparatus, add a small piece of paraffin or a pinch of tannin to prevent foaming, and adjust the tube in place within the flask, which contains 150 cc. of recently boiled water. A few beads or bits of granulated zinc may be added to the water to promote steady boiling. Connect with a condenser and distill by heating the outer flask. Distill 100 cc. and titrate as in *a*.

Notes.—There are various forms and sizes of Sellier and Hortvet distilling tubes in use, but variations in this respect do not apparently affect the result so much as might be expected. The aliquot used is small, and it is virtually impossible to secure results much closer than ±0.006 gram of acetic acid per 100 cc. of wine. The larger apparatus, using 20 cc., is somewhat of an improvement in this respect.

The fixed acids may be determined, if desired, on the same sample by cooling the flask after the distillation is completed,

[1] HORTVET: *Ind. Eng. Chem.*, **1909**, 31.

[2] A somewhat more elaborate form of this apparatus, using a copper outside flask and a constant flow of water, has been described by Gore, U. S. Dept. Agr., *Bur. Chem. Circ.* **44.**

thus draining the wine residue into the larger flask, rinsing with boiled water and titrating as under Total Acids, *b*, page 525.

c. Indirect Method.—If the apparatus for methods *a* and *b* is not available approximate results may be obtained by the indirect method.[1] Evaporate 25 cc. of the wine in a porcelain dish on the water bath to about 3 to 5 cc., dissolve the residue in 25 cc. of hot water, again evaporate to 3 to 5 cc., and repeat once more, making three evaporations in all. Finally, dissolve the residue in hot water and titrate the acids as described under Total Acids, *a*, page 525. From the difference between this titration and the total acids calculate the volatile acids.

Note.—The indirect method in general is inexact on account of the decomposition of acid salts and change of acids during the heating. The modification described here, in which the sample is never heated above the temperature of the water bath and not evaporated to dryness, minimizes these errors and gives fairly concordant and satisfactory results.

Fixed Acids.—These are best determined by difference from the determinations of total acids and volatile acids, although they are determined directly in method *c* for volatile acids above. Calculate in terms of tartaric acid, $H_2C_4H_4O_6$, and if calculating by difference remember that the total acids have been calculated as tartaric and the volatile acids as acetic.

Tartaric Acid and Tartrates. a. Total Tartaric Acid.—The basic principle of the method is the precipitation of both the free and combined tartaric acid as acid potassium tartrate, which is then dissolved and titrated. In the presence of much free tartaric acid, however, the results are low, due, as pointed out by Hartmann and Eoff,[2] to the reversible nature of the reaction, $KCl + H_2C_4H_4O_6 \rightleftharpoons HKC_4H_4O_6 + HCl$, by which a portion of the tartaric acid is not precipitated, the extent of the error being dependent on the amount of free tartaric acid present. This is the reason for neutralizing and adding a known amount of tartaric acid in order to have a definite excess, also for the addition of potassium chloride and acetic acid, thus offsetting the decomposing action of the hydrochloric acid. Under these conditions 1 cc. of $0.1N$ alkali is equivalent to 0.015 gram of tartaric acid. The temperature at which the reaction mixture

[1] WINDISCH: *Z. Nahr.-Genussm.*, **1905**, 70.

[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **162**, p. 71.

is held during the 15 hours is also of great importance, since the lower the temperature the more tartaric acid is precipitated. Hartmann and Eoff propose the following procedure.

Procedure.—Neutralize 100 cc. of the wine exactly with normal sodium hydroxide, calculating the necessary amount from the determination of acidity. If the addition of the normal alkali increases the volume of the solution more than 10 per cent, evaporate it to approximately 100 cc. Add pure tartaric acid, recrystallized, if necessary, to ensure its purity, using 0.075 gram for each cubic centimeter of normal alkali added.

After the tartaric acid is completely dissolved add 2 cc. of glacial acetic acid and 15 grams of potassium chloride. Stir until the potassium chloride is dissolved. The acid potassium tartrate usually begins to form before the potassium chloride is all dissolved, but these two salts are readily distinguished from each other. Add 15 cc. of 95 per cent alcohol, stir vigorously for 5 minutes, and let stand at least 15 hours in an ice box at 15 to 18°C. Then decant the solution through a very thin film of asbestos in a Gooch crucible, wash the precipitate from the beaker with the filtrate (keep cold), and rinse the beaker with a mixture of 15 grams of potassium chloride, 20 cc. of 95 per cent alcohol and 100 cc. of water, making three washings of 7 cc. each. With care, the beaker, precipitate, and crucible may be thoroughly freed from acetic acid with these washings. Transfer the precipitate and asbestos with hot water to the original beaker, using about 50 cc. of water in all, bring to a boil, and titrate with $0.1N$ sodium hydroxide and phenolphthalein.

The grams of tartaric acid in 100 cc. is found from the following:

$$X = [(A + 1.5) \times 0.015] - T,$$

where A is the cubic centimeters of $0.1N$ alkali used for titration, and T is the grams of tartaric acid added.

Note.—1.5 is an allowance for the solubility of the precipitate.

b. Cream of Tartar.—Ignite 25 or 50 cc. of the wine cautiously to a white ash and determine the alkalinity of the soluble ash as described on page 25. Calculate as potassium acid tartrate, $HKC_4H_4O_6$.

c. Free Tartaric Acid.—Add 25 cc. of $0.1N$ hydrochloric acid to the ash of 25 or 50 cc. of wine, heat to incipient boiling, and titrate with $0.1N$ sodium hydroxide, using phenolphthalein as

indicator. Multiply the number of cubic centimeters of standard acid consumed by 0.015 to obtain the equivalent in grams of tartaric acid and subtract this (expressed as grams per 100 cc.) from the result obtained under **a** to obtain the free tartaric acid.

Notes.—Methods **b** and **c** are based on the assumption that by ignition the potassium acid tartrate is converted to an equivalent amount of potassium carbonate, while the free tartaric acid burns to volatile products. It is necessary in method **c** to determine the alkalinity of the total ash, since a small amount of the tartaric acid may be present as an alkaline earth tartrate which would leave an insoluble carbonate.

The relation of the different forms in which the tartaric acid may be present, as calculated from the titration, has been well summarized by Fresenius and Grünhut.[1] If A represents the corrected acidity of the acid potassium tartrate obtained in the determination of total tartaric acid (page 528),[2] G the alkalinity of the total ash, and W the alkalinity of the water-soluble ash, all expressed as cubic centimeters of normal alkali per 100 cc. of the wine, then

(a) If A is greater than G,

Total tartaric acid	=	$0.15A$ [3]
Cream of tartar	=	$0.1881W$ [3]
Alkaline earth tartrates	=	$0.15 (G - W)$
Free tartaric acid	=	$0.15 (A - G)$

(b) If A is equal to or smaller than G, but greater than W,

Total tartaric acid	=	$0.15A$
Cream of tartar	=	$0.1881W$
Alkaline earth tartrates	=	$0.15 (A - W)$
Free tartaric acid	=	0.0

(c) If A is equal to or smaller than W,

Total tartaric acid	=	$0.15A$
Cream of tartar	=	$0.1881A$
Alkaline earth tartrates	=	0.0
Free tartaric acid	=	0.0

Glycerol. *a. Dry Wines.*—(With less than 2 grams of sugar in 100 cc.) Follow the method described for the determination

[1] *Z. anal. Chem.*, **1899**, 477; see also *J. Assoc. Off. Agr. Chem.*, **1915**, 132.
[2] To the number of cubic centimeters of $0.1N$ alkali used add 1.5 cc. and divide the whole by 10 to convert it to normal alkali.
[3] Molecular weight of tartaric acid = 150; of acid potassium tartrate = 188.1.

of glycerol in Vinegar, page 434, using 100 cc. of the sample and making only one preliminary evaporation.

b. Sweet Wines.—(With more than 5 grams of extract or 2 grams of sugar in 100 cc.) Heat 100 cc. of the wine in a flask to boiling and treat it with successive small portions of milk of lime until it becomes first darker and then lighter in color and has an odor of alkali. When cool add 200 cc. of 95 per cent alcohol, allow the precipitate to settle, filter and wash with alcohol. Evaporate the filtrate and proceed as described on page 434 under Vinegar.

Notes.—The above is practically the official German method except that the determination of the extracted glycerol by oxidation with potassium bichromate has been substituted for the admittedly inexact weighing of the crude glycerol. The method is tedious in the extreme and in the case of plastered wines or those containing much sugar yields results that are distinctly too high. On account of the importance of the determination many other methods have been proposed to avoid these difficulties. Of these one of the simplest and most satisfactory is to distill the glycerol under reduced pressure.[1] The method is as follows:

Evaporate 30 cc. of wine to about 5 cc. on a sand bath and rub thoroughly with 15 grams of plaster of Paris. Extract the powder thus obtained for 6 hours in a Soxhlet extractor with hot absolute alcohol and heat the extract, after the addition of 10 to 20 cc. of water, until the alcohol is entirely expelled. Transfer the residue to a 100-cc. retort, the tubulus of which is closed by a good cork, through which passes a short glass rod, well lubricated with vaseline. The neck of the retort passes into a short condenser which is connected with a small strong Erlenmeyer flask, from which a tube leads to a manometer and an efficient pump (Fig. 81). Heat the retort in a small sheet-iron air bath (at ordinary pressure) at 150 to 170°C. until the water is driven off, then connect with the pump and raise the temperature to 190 to 210°C. After the distillation, which usually requires about 1 hour, remove the flame, cool the air bath by removing the cover, and disconnect the pump. In order to recover the glycerol which has condensed in the neck of the retort, add

[1] von Törring: *Z. anal. Chem.*, **1889,** 363; Suhr: *Arch. Hyg.*, **1892,** 305; Partheil: *Z. anal. Chem.*, **1896.** 389.

3 to 5 cc. of water through the cork, again insert the glass rod, and distill, without the pump and with the water drawn from the condenser. In the distillate determine the glycerol by the Hehner method as described on page 436.

Reducing Sugars.—Measure 100 cc. of wine into a porcelain dish, neutralize exactly with a normal sodium hydroxide solution, calculating the amount needed from the determination of acidity, and evaporate on the water bath to about 25 cc. Wash into a 100-cc. flask, add sufficient neutral lead acetate to clarify, fill to the mark, mix thoroughly, and filter through a dry filter. Remove the lead from the filtrate with potassium oxalate as described on page 293, filter, and in an aliquot part of the filtrate
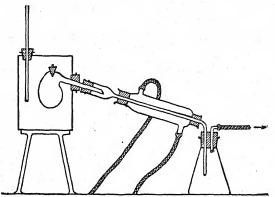
Fig. 81.—Apparatus for glycerol determination.

determine the reducing sugar by one of the methods given on pages 263 to 275. Calculate the result as invert sugar.

Notes.—The aliquot portion taken for the determination should be such that the limits stated on page 269 are not exceeded. This may be calculated approximately by assuming 2 grams per 100 cc. as the sugar-free extract of normal wine.

It will probably be found advisable to redissolve the cuprous oxide and determine it by one of the methods given on page 274 *et seq.* Cane sugar, which is occasionally present, may be determined if desired by determining also the reducing sugar after inversion (see page 275).

If numerous determinations are to be made, a volumetric method, as the Lane and Eynon, is to be preferred for the sake of rapidity; whereas for an occasional test the Munson and Walker,

with or without subsequent determination of the precipitated copper, depending upon the accuracy desired, might be used. An accuracy of 0.02 per cent is ordinarily desirable.

Polarization.—Polarize a part of the lead-free filtrate obtained in the preceding method in a 200-mm. tube. Express the result as the polarization of the undiluted wine in terms of the Ventzke scale (see page 287).

Note.—The polarization of normal wine is ordinarily to the left. If a right-handed polarization is found it may be due to unfermented cane sugar or commercial glucose. In the former case the polarization will be to the left after inversion, in the latter it will probably still be to the right. For the further examination of such a sample to detect the presence of the unfermented constituents of commercial glucose see pages 297 and 333.

Potassium Sulphate.—Acidify 50 or 100 cc. of the wine with hydrochloric acid, heat to boiling, and precipitate with a slight excess of hot barium chloride solution. Let stand for ½ hour, filter, wash, and ignite the barium sulphate in the usual manner. Calculate as potassium sulphate.

Note.—There is always a small amount of potassium sulphate present in grape juice, and a small quantity of sulphuric acid results from the oxidation of part of the sulphur dioxide used in sulphuring the casks, so that the wine will contain on an average about 0.1 per cent, calculated as potassium sulphate. Any excess over 0.2 per cent, is usually accepted as evidence of plastering.

Tannin and Coloring Matter.—Dealcoholize 100 cc. of wine by evaporation and dilute with water to the original volume. On 10 cc. of the solution determine the oxidizable material as described under Cloves, page 411. Call the number of cubic centimeters of permanganate used a.

Treat 10 cc. of the dealcoholized wine, prepared as above, with boneblack[1] for 15 minutes; filter and wash the boneblack thoroughly with water. Add 750 cc. of water and titrate as before. Call the number of cubic centimeters of permanganate used b.

Then $a - b$ = the cubic centimeters of permanganate required to oxidize the tannin and coloring matter in 10 cc. of wine.

[1] Boil 100 grams of finely powdered boneblack with successive portions of hydrochloric acid $(1 + 3)$, filter, and wash with boiling water until free from chlorides. Keep covered with water.

The tannin equivalent of permanganate usually taken for wine is 0.00416, that being the value found by Neubauer for gall tannin, *i.e.*, 1 cc. $0.1N$ permanganate $= 0.00416$ gram tannin.

Note.—In addition to alcohol the wine contains also certain non-volatile substances that are capable of reducing permanganate. The alcohol is removed by evaporation and the tannin and coloring matter are retained by the boneblack, while the other oxidizable substances are determined in the second titration.

Sulphurous Acid and Sulphites.—Use 50 to 100 cc. of wine and carry out the determination as described on page 112 under Preservatives.

Other Preservatives.—The preservatives other than sulphurous acid, which may occasionally be found, include salicylic acid, boric acid, sodium benzoate, and saccharin. In Europe the use has been reported also of cinnamic acid, abrastol, and hexamethylenetetramine.

Salicylic Acid.—Since some genuine wines have been reported as giving tests for a small quantity of salicylic acid the precaution should be taken of not employing too large a sample. Concord grapes have been reported to contain 3.2 mg. of salicylic acid per 100 cc. of juice. Use 50 cc. and carry out the test as described on page 108. If a positive test is obtained and doubt exists as to its being due to added salicylic acid, resort may be had to the quantitative determination.

Benzoic Acid and Benzoates.—Follow the method given on page 104.

Boric Acid.—Boric acid is a normal constituent of wine, so that tests, to have any significance, should be quantitative. To 100 cc. of wine add sodium hydroxide until slightly alkaline, ignite to a white ash, and proceed as directed on page 110.

Saccharin.—See page 121.

Abrastol.—This substance, known also as *asaprol*, is the calcium salt of β-naphtholsulphonic acid. It has been reported used as a preservative in wine and also to take the place of gypsum in plastering. To detect it[1] boil 200 cc. of the wine with 8 cc. of concentrated hydrochloric acid for 1 hour under a reflux condenser. β-naphthol splits off and can be detected by extracting with 10 cc. of chloroform and heating the extract a few minutes with a little alcohol and several pieces of potassium hydroxide.

[1] Sanglé-Ferriere: *Compt. rend.*, **1893**, 796; *Rev. intern. falsif.*, **1894.** 15.

In the presence of β-naphthol a deep-blue color forms, rapidly changing to green and yellow. If only a small amount of abrastol is present the solution may be greenish and only the pieces of alkali colored blue.[1]

Cinnamic Acid.—This may be detected by shaking out a portion of the ether solution obtained in the test for benzoic acid (page 104) with 5 cc. of dilute (0.25N) sodium hydroxide. Heat on the water bath with a few drops of a 5 per cent potassium permanganate solution. With 0.01 mg. or more of cinnamic acid a distinct odor of benzaldehyde will be noticed[2] (see also page 105).

Hexamethylenetetramine.—This, under the name *Urotropin*, has been recommended for removing sulphites from wine. It may be detected by distilling the wine with phosphoric or sulphuric acid, by which the hexamethylenetetramine is decomposed into ammonia and formaldehyde. The latter will appear in the first portion of the distillate and may be identified by the tests given in Chap. III. This method, of course, does not distinguish the hexamethylenetetramine from formaldehyde itself. According to Rosenthaler and Ungerer[3] the most delicate test for hexamethylenetetramine itself is a 5 per cent solution of mercuric chloride, which will give characteristic crystals with 1 part in 500,000.[4] Treat white wines with the reagent directly, after acidifying with hydrochloric acid; treat red wines with dry basic lead acetate, filter, remove the excess of lead with sodium phosphate, and apply the test.

Artificial Color.—Red wines are the only ones that need to be examined for artificial color, since with white wines practically the only additions made to change the color are such substances as caramel, chicory extract, and possibly minute traces of one or two special coal-tar dyes. With red wines a general idea of the likelihood of artificial color being present may be gained by applying the following simple preliminary tests:

a. Basic Lead Acetate Test.—Add to 25 cc. of wine 5 cc. of basic lead acetate solution, shake and note the color of the precipitate. Natural wines impart to the precipitate a gray, bluish-green

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[1] WOLFF: *Pharm. Ztg.*, **1895**, 44.

[2] VON D. HEIDE and JAKOB: *Z. Nahr.-Genussm.*, **1910**, 145.

[3] *Pharm. Zentralhalle*, **1913**, 1153.

[4] See also CHAMOT and MASON: "Handbook of Chemical Microscopy," 2d ed., Vol. II, p. 395.

or green color; certain vegetable colors, like pokeberry or bilberry extract, give a purple color. Add 5 cc. more of the basic lead acetate solution, heat the mixture and filter. Natural wines will, in general, give a very pale or nearly colorless filtrate; if the filtrate is colored distinctly red and yields a red color to amyl alcohol when shaken with it, an artificial color may be present.

b. Mercuric Oxide Test.[1]—Add to 10 cc. of the wine 0.2 gram of yellow mercuric oxide, shake violently for at least 1 minute and after settling filter through a double, wet filter. If the filtrate is not perfectly clear repeat the test, warming the mixture before shaking.

A clear, colored filtrate indicates the presence of a coal-tar color, but, on the other hand, a colorless filtrate should not be taken as necessarily implying the absence of such color, since Erythrosin and Eosin, for example, are absorbed by the mercuric oxide. Other colors, as Orange I and Safranin, are partly absorbed, so that if present in slight amount they might escape detection. Such colors as Bordeaux Red, Amaranth, and Croceine Scarlet are readily shown by this test.

c. Formaldehyde Test.[2]—To 50 cc. of wine add 1 cc. of formalin (40 per cent formaldehyde) and 4 cc. of hydrochloric acid and heat on the water bath until a precipitate forms. Then make slightly ammoniacal and continue the heating until the ammonia is expelled, cool, and filter. Natural wines will, in general, give a colorless filtrate in this test, while the converse is true of artificially colored samples. Like the other simple tests, however, the results must be interpreted with some caution, since it would hardly be safe to assume without further testing in the case of a colorless filtrate that all artificial colors were absent, and further, it has been shown[3] that the test fails with a few very deeply colored genuine wines.

If the results of the preliminary tests indicate the possible presence of an artificial color, a further careful study should be made by the methods outlined in Chap. III, especially the double dyeing method on wool and the examination of the color

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[1] CAZENEUVE: *Compt. rend.*, **1886**, 52; SPAETH: *Z. Nahr.-Genussm.*, **1899**, 633.

[2] JEAN and FRABOT: *Ann. chim. anal.*, **1907**, 52.

[3] ASTRUC: *Ann. chim. anal.*, **1907**, 140.

extracted by amyl alcohol from the acid and the ammoniacal solution.

The older textbooks give complicated procedures for the detection of many vegetable colors, but the reactions are in many cases inconclusive and require much experience for their proper interpretation.

Interpretation of Results

The examination of a wine may be made to determine whether it is true to name or contains any deleterious or forbidden substance, in a word, if it is free from adulteration; or the examination may be to determine the soundness, quality, or capacity for improvement on keeping. An examination for the second purpose demands on the part of the analyst a wide experience with the class of wines in question, and in most cases a trained sense of taste is of as much assistance in forming a judgment as chemical tests. For this reason, the discussion here taken up will necessarily be limited to the detection of adulterations, and for that matter, to certain comparatively gross forms of sophistication and only as they can be shown with some degree of certainty by chemical analysis.

In the customary wine analysis, then, the questions to be answered are such as: Has any improper treatment been employed, or forbidden substance added, during the manufacture of the wine? Is the wine of the character or type claimed by the label? Has any foreign substance, sugar, water, alcohol, etc., been added to the product or during the fermentation?

Analyses of Authentic Samples.—Since European countries were the originators of the modern wine industry and their products still command the highest reputation, it is only to be expected that most of the published analyses of wine are by French and German chemists. Based on these analyses, certain broad standards have been laid down for genuine wines produced by the fermentation of normal grape musts. Even with the extended study that has been given to the subject, these standards, however, are by no means of universal application in European practice, and, when applied to American wines, which are the ones with which we are most concerned, they can be considered only general guides. This difference in the American product is due to the different varieties of the wine grapes

TABLE 85.—ANALYSES OF AMERICAN WINES RECEIVING AWARDS AT PARIS, 1900

(Grams per 100 cc.)[1]

Determination	Sparkling wines			Dry white wines			Dry red wines			Sweet white wines			Sweet red wines		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
pecific gravity 15.5°C	1.0169	0.9910	1.0045	0.9939	0.9901	0.9917	0.9969	0.9926	0.9943	1.0494	0.9908	1.0298	1.0522	1.0107	1.0276
cohol	12.06	9.25	10.48	11.31	8.41	10.17	12.22 / 10.71	8.01	10.00	17.10	9.21	14.53	17.50	10.87 / 13.85	15.31
lycerol	0.7330	0.2301	0.4177	1.0119	0.5690	0.7019	0.9504	0.5341 / 0.5940	0.6355	0.7350 / 0.5924	0.0483 / 0.1316	0.3025	0.7460	0.2936	0.5089
ycerol-alcohol ratio				9.2:100	5.7:100	7.1:100	8.1:100	5.8:100 / 5.1:100	6.4:100						
xtract	8.56	1.78	5.40	2.51	1.55	1.99	3.22	1.77 / 2.02	2.57	19.35	2.83 / 5.71	13.80	19.71	7.57 / 10.69	13.52
h	0.290 / 0.171	0.114	0.153	0.270	0.107 / 0.160	0.196	0.393	0.138	0.247	0.263	0.097	0.203	0.374	0.234	0.311
xtract-ash ratio	1:30.1 / 1:16.9	1:8.5	1:14.6	1:16.4 / 1:13.0	1:6.7	1:10.3	1:18.6 / 1:14.8	1:6.5	1:10.6	1:287 / 1:15.6	1:4.5 / 1:8.8	1:15.0	1:16.6	1:8.4	1:11.1
tal acids	0.783	0.601	0.658	0.715	0.433	0.586	0.901	0.454	0.649	0.805 / 0.534	0.160 / 0.201	0.412	0.826	0.397	0.502
xed acids	0.715	0.418	0.566	0.570	0.326	0.459	0.634	0.346	0.507	0.528 / 0.222	0.116 / 0.029	0.300	0.546 / 0.508	0.257	0.472
latile acids	0.148	0.049	0.082	0.174	0.050	0.101	0.266	0.071	0.128	0.124	0.056 / 0.038	0.092	0.255	0.080	0.122
tal tartaric acid	0.357	0.163 / 0.205	0.271	0.352 / 0.273	0.059 / 0.119	0.189	0.252	0.083	0.163	0.296	0.067	0.142	0.145 / 0.102	0.025	0.078
e tartaric acid	0.141	0.000	0.065	0.1785	0.0000	0.0677	0.0468 / 0.000	0.000	0.000	0.1436	0.000	0.0166	0.000	0.000	0.000
latile acid-total acid ratio	1:14.50	1:4.07	1:9.21	1:9.88	1:383	1:6.21	1:7.90	1:3.38	1:5.71	1:9.47 / 1:6.69	1:2.96 / 1:3.53	1:4.93	1:5.78	1:3.24	1:4.39
arization, °V	−18.3 / −8.0	−1.8	−5.2	−1.1	−0.1	−0.5	−0.8	0.0	−0.4	−25.7 / −14.2	−8.8	−11.8	−19.4	−7.7 / −15.6	−16.4
ducing sugars	5.228	0.023 / 2.390	3.409	0.328	0.051	0.134	0.280 / 0.214	0.045	0.146	16.91	1.76 / 3.71	11.30	16.96	3.24 / 7.66	10.26
tein	0.698 / 0.222	0.070	0.214	0.337 / 0.235	0.060 / 0.109	0.162	0.305	0.077	0.150	0.245	0.026 / 0.105	0.162	0.368 / 0.281	0.105	0.232
assium sulphate	0.128 / 0.047	0.015	0.033	0.130	0.026	0.087	0.133	0.016 / 0.045	0.070	0.088	0.007 / 0.021	0.044	0.062	0.024	0.048
sphoric acid	0.026	0.012	0.017	0.066	0.010	0.022	0.044	0.015	0.029	0.059	0.008	0.034	0.068	0.018 / 0.038	0.045
e sulphurous acid	0.0456 / 0.0051	0.0010	0.0074	0.0078	0.0002	0.0017				0.0188 / 0.0034	0.0011	0.0045			
al sulphurous acid				0.0163	0.0013	0.0063				0.0662 / 0.0445	0.0213	0.0363	0.2207 / 0.1371	0.0516	0.0962
min	0.066	0.009	0.035	0.0830 / 0.0641	0.0178	0.0391	0.3435	0.1388	0.2364						

[1] In some cases the next to the extreme value is given also.

grown in this country and partly to different soil and climatic conditions. It is undoubtedly due also to somewhat less finished methods of manufacture, so that as American winemakers gain experience there is less divergence to be expected between their product and the wines of Europe. This has already been noted in comparing analyses of American wines with those of an earlier period.[1]

A compilation of several thousand reported analyses of wines, chiefly of European origin, will be found in König's "Chemie der menschlichen Nahrungs- und Genussmittel." A somewhat similar compilation comprising, of course, a much more limited number of samples has been made of American wines by Bigelow.[2] Neither of these will be found of the greatest help to the beginner in wine analysis, however; the first because there are so many

TABLE 86.—COMPOSITION OF TYPICAL AMERICAN WINES[1]
(Results are in grams per 100 cc. unless otherwise stated)

	I	II	III	IV	V	VI
Variety[2]	Ives	Clinton-Ives	Dela-ware	Catawba	Zinfan-del	Semillon-Sauterne
Locality	N. Y.	N. Y.	N. Y.	N. Y.	Calif.	Calif.
Alcohol, per cent by volume	11.1	9.18	11.75	8.87	13.9	11.95
Extract	2.32	2.81	1.82	2.52	3.18	2.34
Ash	0.138	0.214	0.133	0.163	0.366	0.327
Alkalinity water-soluble ash, cc. 0.1N acid	8.0	9.3	10.6	12.4	27.1	11.2
Alkalinity water-insoluble ash, cc. 0.1N acid	11.0	12.1	8.6	12.6	12.2	9.2
Total acid as tartaric	0.905	1.087	0.675	1.470	0.551	0.375
Total tartaric acid	0.475	0.488	0.289	0.641	0.146	0.161
Potassium acid tartrate	0.150	0.175	0.199	0.232	0.182	0.201
Free tartaric acid	0.190	0.166	None	0.265	None	None
Tartaric acid combined with alkaline earths	0.165	0.181	0.129	0.189	......	......
Volatile acids as acetic	0.100	0.041	0.075	0.034	0.066	0.122
Volatile esters as ethyl acetate	0.039	0.011	0.037	0.009	0.017	0.020
Aldehyde	0.009	Trace	0.0005	Trace	0.0005	None
Color and tannin	0.226	0.250	0.018	0.042	0.288	0.045
Reducing sugar as dextrose	0.122	0.272	0.076	0.097	0.450	0.460
pH	2.88	2.9	3.13	2.62	3.57	3.28

[1] NELSON and WHEELER: *Ind. Eng. Chem.*, **1939**, 1279.

[2] Zinfandel is a red wine grape giving a "claret" type wine; Ives and Clinton are also native red wines; while Delaware and Catawba are white wines from native grapes, the latter of rather pronounced flavor; and Semillon-Sauterne is from a white grape of superior wine-making quality introduced from France.

[1] U. S. Dept. Agr., *Bur. Chem. Bull.* **72**, p. 22.

[2] U. S. Dept. Agr., *Bur. Chem. Bull.* **59**.

analyses of wines from different sources that one is at a loss as to which to choose for representative figures, and the second because the analyses are taken from all parts of the country, made at different times by different analysts, and admittedly include some not of known purity.

For this reason it has seemed best to limit the analyses given here to the maximum, minimum, and average figures found in Table 85. These are compiled from the analyses of American wines which received awards at the Paris exposition of 1900 and should therefore be typical of the highest grades of genuine American wines. Further, the analysis made was more thorough than in many other published analyses.[1] It should perhaps be added that these figures are given simply to show the range of values to be expected in the various types of American wines, and are not at all to be considered as limiting values for the pure product. A few additional, more recent, analyses of typical American wines, about a year old, are given in Table 86.

The California wines have a considerably smaller percentage of tartaric acid than the New York wines, and they do not contain tartaric acid in a free state.

Federal Standards.[2]—1. *Wine* is the product made by the normal alcoholic fermentation of the juice of sound, ripe grapes and the usual cellar treatment, and contains not less than 7 or more than 16 per cent of alcohol, by volume, and, in 100 cc. (20°C.), not more than 0.1 gram of sodium chloride nor more than 0.2 gram of potassium sulphate, and for red wine not more than 0.14 gram, and for white wine not more than 0.12 gram of volatile acids produced by fermentation and calculated as acetic acid. (*a*) *Red wine* is wine containing the red coloring matter of the skins of grapes. (*b*) *White wine* is wine made from white grapes or the expressed fresh juice of other grapes.

2. *Dry wine* is wine in which the fermentation of the sugars is practically complete and which contains, in 100 cc. (20°C.), less than 1 gram of sugars, and for dry red wine not less than 0.16 gram of grape ash and not less than 1.6 grams of sugar-free grape solids, and for dry white wine not less than 0.13 gram of

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[1] The details of the analyses and description of the samples may be found in *Bur. Chem. Bull.* **72.**

[2] U. S. Dept. Agr., Service and Regulatory Announcements, *Food and Drug No.* 2, Fifth Revision, November, 1936.

grape ash and not less than 1.4 grams of sugar-free grape solids.

3. *Fortified dry wine* is dry wine to which brandy has been added but which conforms in all other particulars to the standard of dry wine.

4. *Sweet wine* is wine in which the alcoholic fermentation has been arrested and which contains, in 100 cc. (20°C.), not less than 1 gram of sugars, and for sweet red wine not less than 0.16 gram of grape ash, and for sweet white wine not less than 0.13 gram of grape ash.

5. *Fortified sweet wine* is sweet wine to which wine spirits have been added. By act of Congress, "sweet wine" used for making fortified sweet wine and "wine spirits" used for such fortification are defined as follows (sec. 43, act of Oct. 1, 1890, 26 Stat. 621; as amended by sec. 68, act of August 27, 1894, 28 Stat. 568; as amended by sec. 1, act of June 7, 1906, 34 Stat. 215; as amended by sec. 2, act of Oct. 22, 1914, 38 Stat. 747; as amended by sec. 402(c), act of Sept. 8, 1916, 39 Stat. 785; and as further amended by sec. 617, act of Feb. 24, 1919, 40 Stat. 1111):

"That the wine spirits mentioned in section 42 is the product resulting from the distillation of fermented grape juice to which water may have been added prior to, during, or after fermentation, for the sole purpose of facilitating the fermentation and economical distillation thereof, and shall be held to include the products from grapes or their residues commonly known as grape brandy and shall include commercial grape brandy which may have been colored with burnt sugar or caramel; and the pure sweet wine, which may be fortified with wine spirits under the provisions of this act, is fermented or partially fermented grape juice only, with the usual cellar treatment, and shall contain no other substance whatever introduced before, at the time of, or after fermentation, except as herein expressly provided: *Provided,* That the addition of pure boiled or condensed grape must or pure crystallized cane or beet sugar, or pure dextrose sugar containing, respectively, not less than 95 per centum of actual sugar, calculated on a dry basis, or water, or any or all of them, to the pure grape juice before fermentation, or to the fermented product of such grape juice, or to both, prior to the fortification herein provided for, either for the purpose of perfecting sweet wines according to commercial standards or for mechanical purposes, shall not be excluded by the definition of pure sweet wine aforesaid; *Provided, however,* That the cane or beet sugar, or pure dextrose sugar added for

sweetening purposes shall not be in excess of 11 per centum of the weight of the wine to be fortified: *And provided further*, That the addition of water herein authorized shall be under such regulations as the Commissioner of Internal Revenue, with the approval of the Secretary of the Treasury, may from time to time prescribe: *Provided, however*, That records kept in accordance with such regulations as to the percentage of saccharine, acid, alcoholic, and added water content of the wine offered for fortification shall be open to inspection by any official of the Department of Agriculture thereto duly authorized by the Secretary of Agriculture; but in no case shall such wines to which water has been added be eligible for fortification under the provisions of this act, where the same, after fermentation and before fortification, have an alcoholic strength of less than 5 per centum of their volume."

6. *Sparkling wine* is wine in which the after part of the fermentation is completed in the bottle, the sediment being disgorged and its place supplied by wine or sugar liquor and/or dextrose liquor, and which contains, in 100 cubic centimeters (20°C.), not less than 0.12 gram of grape ash.

7. *Modified wine, ameliorated wine, corrected wine* is the product made by the alcoholic fermentation, with the usual cellar treatment, of a mixture of the juice of sound, ripe grapes with sugar and/or dextrose, or a sirup containing not less than 65 per cent of these sugars and in quantity not more than enough to raise the alcoholic strength after fermentation to 11 per cent by volume.

8. *Raisin wine* is the product made by the alcoholic fermentation of an infusion of dried or evaporated grapes, or of a mixture of such infusion or of raisins with grape juice.

These standards have not, of course, the force of law and are to be regarded, as in other chapters where they have been quoted, simply as extreme values so fixed as to include practically all commercial goods honestly made with no attempt to defraud.

The official definition of wine[1] is practically identical with the above definition and, since the Department of Agriculture is given authority by Congress to frame regulations for enforcing the Food and Drug Act, thereby legally excludes all substances other than those present in the juice of fresh grapes or ordinarily added in the usual cellar treatment. Principally for the benefit

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[1] U. S. Dept. Agr., *Food Inspection Decision* **156.**

of the wines made in Ohio and Missouri, however, certain additions are allowed for the correction of natural defects due to conditions of soil and climate. (See page 521.)

Characteristics of Genuine Wine. *Alcohol.*—The amount of alcohol in natural unfortified wines is usually between 4.5 and 10 grams per 100 cc., although distinctly higher and lower values have been reported. Since the process of fermentation does not yield over 14.5 grams, values much in excess of this may be considered evidence of added alcohol.

Glycerol.—The glycerol content of normal wines is usually placed at 0.4 to 1.0 gram per 100 cc., although results as low as 0.12 and as high as 1.4 have been reported. There is more variation in this respect in sweet than in dry wines.

Alcohol-Glycerol Ratio $\left(\dfrac{100 \times \text{Glycerol}}{\text{Alcohol}}\right)$.—Since the normal fermentation of sugars with yeast, under restricted conditions, produces a fairly definite proportion of glycerol to the amount of alcohol, this ratio is of much more importance than either alone in deciding whether alcohol has been added to the wine. The former German standard fixed the limits for this ratio between 7 and 14, but the lower limit has been reduced to 6. Even this is rather high for American wines, the minimum ratio in Table 85 being 5.7, and many of the analyses in *Bulletin* 59 showing even lower ratios. Possibly 5.5 would be a fairer standard, although not many of the high-grade domestic wines would fall below the European standard of 6.

Extract.—As in the case of other fermented products in which sugar is the principal substance changed during the fermentation, the sugar-free extract is much more nearly constant than the total extract (see also under Vinegar, page 441), and lends itself much better to the fixing of a minimum standard. The amount of potassium sulphate in plastered wines should also be subtracted. Since wines contain normally a small amount of both sugar and potassium sulphate, a common formula in European practice for determining the "reduced extract," as it is called, is

$$R = E - (S - 0.1) - (K - 0.1),$$

where E is the grams of extract, S the sugar, and K the potassium sulphate per 100 cc. of wine. The reduced extract for white

wines usually varies between 1.5 and 2.6 grams, for red wines between 1.8 and 3.0 grams. The amount of extract decreases slightly as the wine grows older, but will seldom fall below 1.5 grams per 100 cc. Compare also the standards for sugar-free extract given on page 540.

Ash.—The extreme limits that have been suggested for the ash of all wines are 0.11 to 0.44 gram per 100 cc. These extremes are not often met, and fairer limits for usual practice would be set at 0.14 to 0.35 gram. Since these standards are stated in terms of "grape ash," any excess of potassium sulphate over 0.1 gram should be deducted.

Alcohol-Extract Ratio $\left(\dfrac{Alcohol}{Extract}\right)$.—French authorities place the maximum values for this ratio at 4.5 for genuine red wine and at 6.5 for white wines. Higher values than these are considered evidence of added alcohol. The figures are best considered in connection with the Glycerol-Alcohol ratio.

Acidity.—The total acidity, expressed as tartaric acid, usually lies between 0.5 and 0.9 gram per 100 cc., although values of 0.3 and 1.7 have been found.

The volatile acids are more a measure of the care taken in the fermentation and handling of the wine than a criterion of freedom from adulteration. The maximum limit adopted in European countries is 0.12 gram per 100 cc. for normal wines, and values over 0.15 gram are held to indicate an unsound wine. Conditions in this country are such that the values adopted might well be somewhat higher, although these limits are practically those given in the standards on page 540.

Of greater value for the detection of adulteration is the amount of free tartaric acid and its relation to the fixed acids, since normal wine from ripe grapes contains no free tartaric acid, and only a slight amount is found even in wine from unripe grapes. Kunz[1] reports values of 0 to 0.105 gram per 100 cc. (average = 0.025) for free tartaric acid, while other observers have found from 0.1 to 0.3 gram in wines from unripe grapes. The German Commission makes the general rule that the free tartaric acid should not exceed one-sixth of the fixed acid, provided that the amount of total free acids is not more than

[1] *Z. Nahr.-Genussm.*, **1901**, 673.

0.8 gram per 100 cc. Natural wines with higher total acid frequently contain more free tartaric acid.[1]

Detection of Specific Adulterations. *a. Added Alcohol.*—As stated on page 543, the presence of more than 14.5 grams of alcohol per 100 cc. is direct evidence of added alcohol, an addition which is allowable in the case of fortified sweet wines (see page 541). With wines that contain an amount less than 14.5 grams the safest guide is the ratio between the alcohol and glycerol. Wines which contain less than 6 grams of glycerol to 100 of alcohol are generally regarded as having added spirit. On the other hand, wines with very low glycerol content, corresponding to 6 grams or less of alcohol (less than 0.36 gram of glycerol), have probably been made by arresting the fermentation of the must through the addition of alcohol.

A criterion of the presence of added alcohol which is frequently quoted is the alcohol-extract ratio (page 544). A value for the ratio, $\dfrac{\text{alcohol}}{\text{reduced extract}}$, greater than 4.5 for red wines or 6.5 for white wines is considered by French authorities as indicative of added alcohol. The "natural alcohol" is calculated in such a case by multiplying the reduced extract by 4.5 or 6.5 as the case may be.

b. Watering.—The problem of showing added water in wine is almost analogous to the same question in Milk (page 148). The same suggestion has been made, *i.e.*, to show the presence of the water by impurities which it may contain, chiefly by its content of nitrates, but further observations have shown that such methods are not reliable. The only practical method of determining whether water has been added is by comparison with a similar wine of known purity to show whether the characteristic constants, ash, extract, acids, and alcohol, have all been lowered, and in approximately the same proportion.

For French wines the relation between the acids and the alcohol is considered important for showing watering. According to Gautier,[2] in normal wine the amount of alcohol varies inversely as the free acids, the sum of the two being nearly a constant. The usual form adopted for expressing this relation is to add to the per cent of alcohol by volume the total acid,

[1] Kulisch: *Chem.-Ztg.*, 1908, 1105.

[2] "Traité sur la sophistication et l'analyse des vins."

calculated as grams of sulphuric acid per liter. A value for the figure thus obtained below 13 for red wines or below 12 for white wines is considered to indicate added water.

If the wine has been shown, according to *a*, to contain added alcohol, the value used in the above calculation should not be the per cent of alcohol as found but the per cent by volume corresponding to the "natural alcohol," calculated as previously described.

c. Plastering.—Additions of gypsum in such quantity as to raise the sulphuric acid content of the wine, expressed as potassium sulphate, over 0.2 gram per 100 cc. are definitely forbidden by the wine laws of European countries, and the same limit is incorporated in the Federal standards (page 540). Smaller additions than this are best shown in the increased sulphuric acid in the ash. Natural wines do not ordinarily contain more than 0.01 to 0.014 gram of sulphuric acid (SO_3), corresponding to 3.8 to 25 per cent (average, 10.5 per cent) of the ash, while the ash of plastered wines frequently contains 40 per cent or more. There is also a corresponding decrease, in the alkalinity and carbonic acid content of the ash.[1]

d. Sugared Wines.—The European wine-producing countries are inclined to look with disfavor upon the addition of sugar, either dry or as a solution, to wine. Under exceptional circumstances the addition of small quantities of pure dry sugar may be permitted if necessary to correct defects in the product. An extension of this practice is the addition to the must of a sugar solution or sugar sirup (*gallization*). Such products, however, cannot be sold under the name "wine" without qualification.[2]

1. *Addition of Dry Sugar (Sucrose).*—If the added sugar has not been entirely fermented, a qualitative test for its presence will be sufficient in case of a positive result to show that sugar has been added, since natural wines contain no sucrose. A test frequently used to show this is that of Rothenfusser.[3]

[1] CARPENTIERE: *Z. Nahr.-Genussm.*, **1912**, 42.

[2] See in this connection, Ameliorated Wine, p. 542, and U. S. Dept. Agr., *Food Inspection Decision* **156**, as quoted on p. 521.

[3] *Z. Nahr.-Genussm.*, **1909**, 135. Heat 25 cc. of wine on the water bath to 85 to 90°C. Add an equal volume of a freshly prepared mixture of 2 parts of lead acetate (500 grams in 1,200 cc. water) and 1 part of ammonia

If the sugar added was anhydrous grape sugar (glucose) and it has not all been fermented, its presence will ordinarily be shown by the determination of reducing sugar and polarization. Any residual unfermented sugar in a natural wine is mainly levulose, since the dextrose is more readily fermented. In fresh musts the ratio of dextrose to levulose has been observed to be 100:77 to 84; after a week's fermentation, however, the wine contained 1½ to 6 times as much levulose as dextrose.[1] In natural wine, then, the polarization would be to the left, while the presence of an appreciable amount of anhydrous grape sugar, dextrose, or corn sugar, would cause it to be to the right.

The addition of commercial invert sugar is much harder to detect, but, even here, if the added sugar has not completely fermented it will be found that in natural wines the polarization is more strongly levorotatory, as compared with the total reducing sugars, than would be the case with invert sugar itself, the difference being due again to the preponderance of levulose in the genuine product.

If the added sugar has been completely fermented, a clue to the addition may still be gained from the ratio of the acids to the alcohol, since the musts, which are low in sugar and hence would yield little alcohol if no sugar were added, are usually high in acidity. According to Windisch[2] an alcohol content of more than 9 grams per 100 cc. together with over 0.9 gram of total acids is suspicious of added dry sugar.

French authorities frequently calculate the sugar present in the original must in order to determine whether sugar has been added. This is taken as the sum of the sugar present in the wine, and twice the alcohol (since 100 parts of dextrose yield by fermentation approximately 50 parts of alcohol), both expressed in grams per 100 cc. Since it has been determined by many

(sp. gr. 0.94), shake violently for 30 seconds, and filter. Mix 3 cc. of the clear, colorless filtrate with an equal volume of diphenylamine reagent (10 cc. of 10 per cent alcoholic diphenylamine solution, 25 cc. of acetic acid, and 65 cc. of concentrated hydrochloric acid), heat 10 minutes in boiling water and note the appearance of a blue color (due to the formation of fructose). At the same time heat another portion of the filtrate in the same bath with an equal volume of Fehling's solution. If no reduction is observed and the diphenylamine test shows a blue color, sucrose is present.

[1] KÖNIG and KARSCH: *Z. anal. Chem.*, **1895**, 3.

[2] "Die chemische Untersuchung und Beurtheilung des Weines."

analyses that for French musts the sugar content never exceeds 32.5 grams per 100 cc., any excess of the calculated sugar over 32.5 grams is taken as indicating an addition of either sugar or alcohol.

2. *Addition of a Sugar Solution.*—If a portion of the added sugar remains still unfermented it may be detected as mentioned under 1. With thoroughly fermented wines, however, the sugaring must be detected by the lowering of some important values or changing of known ratios. The alcohol content is raised, while on the other hand the percentages of acids, extract, ash, and nitrogen are lowered. This decrease is not necessarily proportional to the dilution by the sugar solution, for according to Kulisch[1] new constituents of the extract, especially glycerol, are formed by the fermentation of the sugar. It is especially noticeable, however, in the ash, nitrogen, and phosphoric acid.

The Swiss "Lebensmittelbuch"[2] fixes the following minimum limits for fixed acids and "extract rest" (see page 549) for natural, unsugared wines for each per cent of alcohol.

Alcohol, per cent. by volume	Fixed acids, grams in 100 cc.	Extract rest, grams in 100 cc.	
		Red wines	White wines
7	0.66	1.05	0.85
8	0.57	1.10	0.90
9	0.50	1.15	0.95
10	0.45	1.20	1.00
11	0.41	1.25	1.05
12	0.38	1.30	1.10
13	0.36	1.35	1.15

The phosphoric acid (P_2O_5) should not be less than 0.008 gram in 100 cc.

e. Preservatives. Sulphurous Acid.—The only preservative tolerated in wine is sulphurous acid. For the detection of other preservatives see pages 534 to 535, noting the occasional presence of small quantities of some of them naturally in wines. The form in which sulphurous acid is supposed to be added is that of the sulphur dioxide itself, as produced ordinarily by burning

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[1] *Arb. kaiserl. Gesundh.-amte,* **1910,** 1.

[2] 3d ed., p. 38.

sulphur, rather than in the form of sulphites, and the amount that is allowed is not to exceed 350 mg. per liter, of total sulphurous acid, of which not more than 70 mg. shall be in the free state.[1] In other countries the amount allowed is frequently less, 200 mg. per liter and 50, 20, or even 16 mg. of the free acid, being variously set as the limits.[2]

f. Pomace Wine.—The following table[3] illustrates the difference in composition between a genuine wine and the so-called "pomace" wines obtained by extracting the marc or pomace several successive times with sugar solutions and fermenting the products.

TABLE 87.—COMPOSITION OF POMACE WINE
(Grams per 100 cc.)

Sample	Ex-tract	Free acids	Cream of tartar	Tannin	Nitro-gen	Ash	Potash (K_2O)	Lime (CaO)	Phos-phoric acid (P_2O_5)
Genuine wine....	2.11	0.78	0.388	0.0122	0.0341	0.222	0.1086	0.0094	0.0205
1st Pomace wine.	1.63	0.49	0.273	0.0165	0.0107	0.217	0.1011	0.0098	0.0109
2nd Pomace wine.	1.22	0.39	0.203	0.0288	0.0025	0.162	0.0846	0.0111	0.0038
3rd Pomace wine.	0.91	0.34	0.158	0.0273	0.0022	0.138	0.0618	0.0131	0.0030
4th Pomace wine.	0.88	0.33	0.063	0.0316	0.0003	0.100	0.0397	0.0160	0.0020

It will be observed that the pomace wines are low in extract, acids, nitrogen, and phosphoric acid, but high in tannin. The ash, especially the potash and lime salts, is usually high but occasionally may be found very low, hence is not so satisfactory as an indicator.

The ratio of the extract to the ash (see page 538) is frequently higher than 10:1 and even as high as 10:2.

The "undetermined extract" or "extract rest," as it is sometimes called, *i.e.*, the extract minus the sum of the fixed acids, glycerol, and ash, according to Fresenius and Grünhut[4] is generally below the normal value for wine, 0.35 gram per 100 cc. When it is higher the increase is usually to be attributed to an abnormally high content of tannin. For this reason it has been suggested[5] to subtract from the extract five times the per cent of

[1] U. S. Dept. Agr., *Food Inspection Decision* **76**.

[2] MASTBAUM: *Chem.-Ztg.*, **1908**, 427.

[3] WEIGERT: *Mitt. d. Versuchsstation Klosterneuberg*, **1888**.

[4] *Z. anal. Chem.*, **1898**, 472.

[5] BARTH: *Z. Nahr.-Genussm.*, **1899**, 106.

tannin. With natural wines the difference should not be below 1.5.

Fresenius and Grünhut[1] state further that more than 0.03 gram of tannin per 100 cc. indicates usually either a pomace wine or the addition of tannin. It is true also that pomace wines have been found with much lower tannin content than this.

It has been claimed also that normal white wines contain about 0.1 gram per 100 cc. of tartaric acid combined with alkaline earths; whereas in pomace wines this value is either extremely low or entirely lacking. This result, however, should be interpreted with considerable caution on account of the variations it may possibly show through excessive sulphuring or by the possible direct addition of organic acids.

The glycerol-alcohol ratio in pomace wines is generally higher than 7:100 and frequently reaches 10:100.

The nitrogen content of natural wines is seldom under 0.007 gram per 100 cc., varying ordinarily between this value and 0.009; whereas for pomace wines the percentage present is very much lower.

The content of tartaric acid is naturally of great importance, the pomace wines usually containing distinctly less of this characteristic constituent. In the case of the *United States vs. 60 Barrels of Wine* (*Notice of Judgment* 3529), the contention was successfully made on behalf of the government that a true Ohio claret wine cannot possibly contain less than 0.2 per cent of total tartaric acid, that the amount present is usually 0.3 per cent, and may be as high as 0.5 per cent. The wine in question, which was adjudged to be a pomace wine, contained 0.05 per cent of total tartaric acid.

A detailed study of pomace wine, to which further reference should be made, has been reported by Eoff.[2]

g. Imitation Wine.—Entirely artificial wines, in whose preparation no grape juice has been used, may be found, although they are not so common as those in which a pomace or base wine has been so manipulated as to imitate a natural wine. These wines may be identified by discrepancies in the relation of the constituents ordinarily determined, since it would be extremely difficult, if not impossible, to prepare a mixture

[1] *Loc. cit.*

[2] *Ind. Eng. Chem.*, **1916**, 723.

which would imitate a true wine in all its characteristics, including that of taste. Further, such a wine would be detected in most cases by the absence of the characteristic constituents, lecithin and inosite, which are almost invariably present in natural wine. Lecithin is present in amounts varying from 0.05 to 0.10 gram per 100 cc.[1] To detect inosite, add to 200 cc. of wine 20 cc. of basic lead acetate solution, and 2 to 3 cc. of an alcoholic solution of tannin, and filter. Remove the excess of lead with hydrogen sulphide; filter, decolorize by boneblack (see page 533), and concentrate to 10 cc. on the water bath. Test a portion by Scheur's test,[2] depending upon the oxidation of inosite with nitric acid to colored oxyquinone derivatives, as follows:

Treat a few cubic centimeters of the concentrated solution with a little nitric acid and evaporate upon the water bath almost to dryness; add a little ammonia and barium chloride solution and again evaporate. A rose-red color will develop in the presence of 0.5 mg. or more of inosite.

h. Fruit Juices.—The addition of fruit juices such as apple, pear, currant, and the like, either directly or in the form of cider, perry, etc., can often be shown by the presence of *sorbitol*. This, an alcohol derived from dextrose, has been found by various observers[3] in apples, pears, plums, dried dates, sultanas, raisins, and Xanti currants, but not in grapes, raspberries, red currants, and cranberries, among other fruits. It may be separated by its condensation with benzaldehyde, forming crystals of benzylidene-sorbitol, which give a color reaction with acetone and sulphuric acid, owing to the benzaldehyde set free. For details of the test and its quantitative determination the original article should be consulted.

Adulterated Samples.—For further study in the interpretation of a wine analysis there have been gathered in Table 88, page 554, analyses of various adulterated samples that have been prosecuted successfully under the Food and Drug Act. In most of the cases the defendants pleaded guilty, which is as good evidence as needed of the correctness of the analyst's judgment of the sample.

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[1] For its detection and estimation see LEACH-WINTON: "Food Inspection and Analysis," 4th ed., pp. 278 and 366.

[2] *Annalen*, **1852**, 375.

[3] REIF: *Z. Unters. Lebensm.* **1934**, 179; *Analyst*, **1934**, 760.

A comparison of the values in the table with those given in *Bulletin* 72 of the Bureau of Chemistry, or the average values in Table 85, will be found most helpful.

The description of the samples follows:

1. This product was labeled: "Select Riesling Wine, Special Vintage, ..." Misbranding was charged on the ground that the label would lead the purchaser to believe that the product was Riesling wine of a select quality, when as a matter of fact the analysis showed it to be a compound of wine and a fermented solution of commercial dextrose, otherwise known as starch sugar.

2. The principal label in this case read: "Special Queen of Lake Erie Ohio Scuppernong Wine . . . ." The product was also "Guaranteed not to be adulterated or misbranded within the meaning of the National Food Law." Adulteration was alleged in this case for the reason that the product was not a true wine made from Scuppernong grapes, but was a mixture of pomace wine and a wine made from grapes other than Scuppernong grapes, and contained very little, if any, genuine Scuppernong wine. The question of the actual presence of Scuppernong wine was of course decided largely by the taste and general appearance of the wine. The analysis is cited here because it is a typical mixture of a pomace with a sweet wine.

3. In distinction from the preceding analysis, this illustrates the adulteration of a *dry* wine with a wine prepared from pomace. The package bore the label: "Ohio Catawba Wine . . . . Special," but the conclusion drawn from the analysis was that the product was not genuine Catawba wine but was prepared in whole or in part from pomace.

In all of these cases cited, the defendants pleaded *nolo contendere*, and a fine was imposed by the court.

4. "Select Scuppernong Wine." From the analysis it was found that this sample was artificially prepared to resemble Scuppernong wine. Adulteration of the product was alleged in the information because of the substitution of a mixture containing sugar, water, flavor, and the juice of grapes other than Scuppernong wholly or in part for the genuine article. Note especially the proportion of tartaric acid and sucrose.

5. This sample is somewhat different from the others tabulated, in that it was represented to be a genuine French cham-

pagne of high quality, "Extra Dry Superior Quality" appearing on the neck label, and "Sparkling Wine Extra Dry Les Etoiles D'Or" on the body label of the bottle. The product was held to be adulterated in that an imitation champagne of domestic origin, made in part from pomace wine and artificially carbonated, had been substituted, in part, for genuine sparkling wine champagne. The defendants pleaded guilty, and a fine was imposed. Compare the analysis carefully with the figures given for sparkling wines in Table 85.

6. On the cases containing the wine appeared the statement: "Sauterne Extra Dry," and on the bottles the labels read: "Sparkling Carbonated Wine Extra Dry Sauterne Type . . . ." By comparison with Table 85 it is seen at once that this is not of the type of Sauterne, which is a dry white wine, and from the reducing sugar content it certainly is not extra dry. An indictment was successfully brought on the grounds that a domestic white wine, artificially carbonated and not extra dry, had been substituted entirely for genuine Sauterne wine.

7. The analysis given for this sample is not so extensive as some of the others, but enough is given to bear out the contention of the chemist that the product, labeled "Old Bass Island Ohio Port," was not port wine but an imitation port wine or an unfinished wine insufficiently fortified with alcohol (see figures for red sweet wines in Table 85, and analyses of American port wines in *Bulletins* 59 and 72 of the Bureau of Chemistry). The defendant company pleaded guilty to the charge and was fined $100 and costs.

8. The final sample of the table bore the label: "Ohio Golden Eagle Sauterne Wine." The analysis shows, however, that the sample does not correspond at all to a dry white wine, but indicates the presence of a considerable proportion of a pomace wine, sweetened and flavored. The makers of the product did not contest the case but paid the fine imposed.

Whisky

As was stated in the opening paragraphs of this chapter, in order to produce a beverage containing a greater amount of alcohol than is possible in a fermented liquor as beer or wine, resort must be had to distillation. The distilled liquors thus produced vary greatly in their flavor and in their general character-

TABLE 88.—ANALYSES OF ADULTERATED WINES (Grams per 100 cc.)

Determination	I	II	III	IV	V	VI	VII	VIII
Specific gravity (15.6°/15.6°C.)		1.0700	0.9901	1.0552	1.0021	1.0132		1.0317
Alcohol (per cent by volume)	12.95	12.91	13.07	12.50	11.77	11.63	7.15	13.12
Total solids (extract)	2.52	22.51	1.78	18.53	4.47	7.07	1.72	12.58
Sugar-free solids	2.12	1.95	1.60	1.87	2.13	2.13		2.41
Glycerol	0.40		0.702			0.42		
Reducing sugar	0.40	20.42	0.175	6.12	2.15	4.86		10.07
Reducing sugar after inversion	0.40							
Sucrose		0.14		10.54	0.18	0.08		0.630
Total acid as tartaric		0.634	0.533	0.596	0.713	0.628	0.495	0.436
Fixed acid as tartaric		0.416	0.388	0.422	0.486		0.456	0.155
Volatile acid as acetic		0.174	0.116	0.139	0.181	0.104	0.031	0.172
Total tartaric acid		0.144	0.165	0.114	0.227		0.335	0.00
Free tartaric acid		0.0	0.021	0.00	0.034		0.137	0.210
Cream of tartar		0.141	0.067	0.122	0.147		0.180	0.00
Tartaric acid combined with alkaline earths		0.030	0.090	0.010	0.075		0.054	
Tannin and coloring matter		0.021	0.019		0.009			
Polarization, direct, at 20°C. (°V.)	+3.5	−5.7	+0.5		−3.0	−0.3		−3.0
Polarization, invert, at 20°C. (°V.)	+3.5	−6.0			−4.0	−0.4		−3.1
Ash		0.122	0.146	0.172	0.194	0.196	0.110	0.192
Alkalinity water-soluble ash (cc. 0.1N acid per 100 cc.)		7.5	3.6	7.0	7.8			11.4
Alkalinity water-insoluble ash (cc. 0.1N acid per 100 cc.)		6.2	6.0	5.6	5.0			12.0
Total phosphoric acid (P_2O_5)		0.0138	0.0136	0.0253	0.0426	0.0117		
Chlorine (Cl)		0.0153	0.0168	0.0548	0.0454			0.0256
Potassium oxide (K_2O)		0.337	0.0349	0.0139	0.0330			
Sodium oxide (Na_2O)		0.0101	0.0050					
Calcium oxide (CaO)		0.0100	0.0134					
Magnesium oxide (MgO)		0.0092	0.0063					
Sulphuric acid (SO_3)		0.0100	0.0276			0.084 (K_2SO_4)		

istics, depending upon the methods by which they are made and the raw materials used. Possibly the most logical one to take as typical would be *brandy*, this being obtained by the distillation of wine or wine residues, which have just been discussed. Brandy is, moreover, probably the highest grade of distilled liquor produced, being made from what is itself the purest fermented product, wine. In this country, however, a more characteristic product is that made by distilling a fermented grain mash, practically by distilling beer, and known as *whisky*. For this reason it has been chosen as representative of this class of food products.

Definition.—Whisky may be defined in a broad sense as a potable spirit obtained by distillation from a fermented grain mash composed of malt alone or of cereal grains saccharified by the diastase of malt.

It is understood in this definition by the use of the term "potable spirit" that the whisky shall contain a certain proportion of the impurities, or, as they are sometimes called, the "congeneric substances," which are produced with the alcohol during the fermentation and distillation and accompany it into the distillate. Indeed, it is mainly to these "impurities" that whisky owes its characteristic flavor; since, if the process of manufacture be so carried out that these are eliminated, so far as possible, the resulting product is a rectified spirit, containing over 95 per cent of pure alcohol, and quite lacking in the taste and characteristics of what we ordinarily term whisky.

This greater elimination of these secondary constituents from the distillate by the efficient modern or so-called "patent" stills has caused in the past much acrimonious discussion as to what the word "whisky" shall actually include, some wishing to limit the term absolutely to the product of the simpler, older type of "pot still" which retains a large proportion of the congeneric substances. On the other hand, it has been contended that the word "whisky" is applicable to all grain distillates, irrespective of the form of still employed. The latter view has been upheld by a British Royal Commission on potable spirits, which declined to limit the term to the product of the pot still, and in this country the word "whisky," without qualification, has been sanctioned for "all unmixed distilled spirits from grain, colored and flavored with harmless color and flavor, in the customary ways."[1]

[1] U. S. Dept. Agr., *Food Inspection Decision* 113.

Whisky may be further distinguished in respect to the particular grain employed, as *rye whisky*, made from a mixture of rye or barley malt and unmalted rye, and *Bourbon whisky*, in the manufacture of which a large proportion of Indian corn (maize) is employed; or according to the country of origin, as American, Scotch, or Irish whisky.

Manufacture.—Only the general outlines of the manufacturing processes can be taken up here, and especially as they may affect the chemical composition of the finished product. For a more detailed description of the methods and apparatus employed reference should be made to the works listed at the end of the chapter. The manufacturing processes group themselves naturally into three divisions: Mashing and Fermenting, Distilling, and Aging.

Mashing and Fermenting.—The finely ground grain, together oftentimes with a small proportion of malt, is heated with water until the starch is "pasted," then cooled, the main bulk of the malt added and the mixture kept at the proper temperature (140 to 150°F.) until the maximum amount of diastatic action has taken place, the object being to convert the starch to maltose as thoroughly as possible. Specially prepared yeast is now added and the fermentation allowed to continue for 3 to 4 days, when the resulting dilute alcoholic liquid or "beer" is ready for distillation.

Distilling.—It is common practice in this country to conduct the distillation in two operations. In the first, a so-called "beer still," acting either intermittently or continuously, is employed to concentrate the alcohol from the beer. In the continuous still, as the name indicates, the process goes on without interruption, the heated beer being added at the top of the still, through which steam is ascending, and the dealcoholized beer or "slop" being drawn off at the bottom. In the operation as conducted intermittently, each charge of the still is distilled as three fractions, the "heads," "middle run," and "tails." The heads and tails are commonly returned to the still and distilled with the next charge, so that the final products are the spent beer or slop, and the "high wines" containing about 60 to 70 per cent of alcohol.

In both cases the product of the first distillation is subjected to a redistillation in a steam-heated pot still, the middle portion

of the distillate, when reduced to proof, constituting the crude or raw whisky.

The distillation may be also carried on by the use of rectifying or column stills, which make use of the principle of fractionation by means of dephlegmators to obtain a more concentrated alcohol, nearly free from the secondary products. A partial rectification may also be secured by passing the distillate through tubes containing charcoal, which removes a portion of the higher alcohols.

Aging.—The whisky when first distilled is of a harsh, unpleasant flavor and is usually stored for some time in order that changes may take place in the secondary constituents which tend to make the product more palatable. The effect of these changes upon the composition of the whisky is discussed later. The storage of the whisky during this period is conducted in oak barrels, much of the change in flavor and practically all the color being as a matter of fact due more to the wood of the barrel than to any chemical change in the whisky itself. In order to increase the extent of the change, it is customary with American whisky to char the interior of the barrels.

All the processes that have been described are carried on under supervision of the Internal Revenue Bureau of the Federal Government, the materials and product being subject to government inspection from the time the grain is weighed for grinding and mashing until the aged whisky is withdrawn for the market. Government officials are stationed at the distilleries for the purpose of enforcing this requirement. When the whisky is placed in the government warehouse to be aged, it is stamped with a "warehouse stamp" which bears the date of inspection and the gauge. When the aged product is withdrawn, the tax is paid and another stamp attesting the fact affixed. This constitutes a "double-stamped package."

For the further protection of the retail purchaser the whisky after storage is allowed to be "bottled in bond." This amounts to a guaranty on the part of the government that the whisky is at least 4 years old, of standard 100° proof, and that nothing has been added to it since it was distilled other than the water that may have been added to reduce it to 100° proof and what substances it may take up itself from the package in which it is kept in the warehouse. This is attested by a suitable seal issued

by the Commissioner of Internal Revenue and placed over the cork before the bottle is allowed to be withdrawn for sale.

The outline of manufacturing processes given above has special reference to whisky made in this country. Scotch and Irish whiskies are made by methods which are generally similar, although there is produced in those countries possibly a somewhat larger proportion of liquor made by the cruder fire-heated pot still. The characteristic Scotch whiskies owe their peculiar smoky flavor and taste to the employment of peat as fuel in drying the malt.

Quick-aging.—Impatient of the slow natural method of maturing liquors, there have always been attempts to hasten the process, a desire especially manifest in the period after the repeal of prohibition because of the exhausted stocks of naturally aged material.

Numerous methods have been proposed for this purpose, both chemical and physical, depending mainly upon oxidation, accelerated by heat, upon electrolysis, or upon the solution in a shorter time of the products taken up from the wood of the barrel by using this in the form of shavings or chips, to mention only a few. Such products are naturally of inferior quality, and it is often one of the tasks of the analyst to detect such treatment. A discussion of these processes and a bibliography of the subject will be found in a paper by Fain and Snell.[1]

General Composition.—The popular conception of whisky as a solution of ethyl alcohol in water, containing an extremely minute quantity of substances that give it a taste and flavor, is as far wrong as the notion, so long held, that the injurious effects of whisky are due mainly if not wholly to its content of "fusel oil." It is true that whisky contains nearly half its volume of alcohol, but in addition it contains a number of other substances present in readily measurable quantities and so variable in amount that whisky is in reality a very complex liquid. As regards the other prevalent belief, it is undoubtedly true that the toxic effects of fusel oil, in the proportion in which it is found in whisky, have been greatly exaggerated, and it is also probably true that the preponderating agent in the whisky which produces physiological effects is the ethyl alcohol.

[1] *Ind. Eng. Chem., News Ed.,* **1934,** 120.

These substances, which are present along with the alcohol, are in one sense impurities and were so termed on page 555, but from the standpoint of flavor they are valuable and necessary constituents. The case is in many ways analogous to maple sirup (see page 307), the principal constituent of which is sugar, but which owes its valued characteristics to small quantities of "impurities" which impart the special flavor for which it is prized. "They are the associated bodies which give the alcohol its special and valued characters, and to their production, modification, or elimination by age we owe the changes which spirits undergo during the process of maturing."[1]

The most important of these secondary constituents comprise: *acids*, principally acetic and valeric, with traces of propionic and others; *esters*, chiefly ethyl acetate and valerate, from the ethyl alcohol, together with amyl acetate and valerate, derived from amyl alcohol; *furfural*, present in small but measurable quantities; *aldehydes*, principally acetaldehyde but, undoubtedly, including small amounts of other aldehydes resulting from the oxidation of the corresponding alcohols; *fusel oil* or "*higher alcohols*," consisting mainly of amyl and isobutyl alcohols, with smaller quantities of isopropyl and normal propyl alcohols and traces of other alcohols, acids, and ethers.

The proportion of the secondary constituents found naturally varies with the character of the materials and the exact methods of fermentation and distillation employed. Some of them, as the higher alcohols and the acids, are formed during the fermentation and hence are present in all whisky in considerable amounts unless removed or decreased by the process of distillation. Others, of which the aldehydes and furfural are typical, are normally produced very largely during the distillation, either by the action of the live steam employed or by the charring of the materials when the still is directly fire-heated, and hence will vary according to the exact apparatus and method used.

The changes that take place in the congeneric substances during the storage or aging of the whisky, as they affect the interpretation of analyses, are discussed on page 579. It will suffice to say here that the popular theory that during the aging the higher alcohols are eliminated or so changed by oxidation

[1] ALLEN, A. H.: *Analyst*, **1891**, 102.

that the whisky loses its harsh and unpalatable character has been shown to be entirely incorrect. As a matter of fact, the proportion of the higher alcohols, as is true of practically all other constituents, is increased during the aging. The disagreeable taste of new whisky has been ascribed by Schidrowitz[1] to the presence of pyrrol, as well as phenolic and sulphur compounds, which are either resinified or oxidized during the aging, so that they are not found in the matured product.

It is only comparatively recently that due consideration has been given to the changes produced in the composition of whisky due to its being stored in charred barrels (see page 579). The storing in charred barrels is in effect a sort of rectification, since the action of the charcoal is not essentially different from the charcoal tubes or tanks used in some direct rectifying processes except that, in this case, we have to deal with the residual liquid rather than what has passed through the charcoal. This is important in itself, to say nothing of the tannin, color, and solids taken up from the wood of the barrel.

The relative amounts of the various secondary constituents that are present vary considerably in different kinds of distilled liquors, rum for example being much higher in esters than is the case with whisky, but the total amount is always much higher the simpler the process, *i.e.*, the less the amount of frac-

TABLE 89.—SECONDARY CONSTITUENTS IN DISTILLED SPIRITS

	Grams per 100 liters *					
	Volatile acids	Esters	Aldehydes	Furfural	Higher alcohols	Total
Genuine gin....................	0.0	37.3	1.8	0.0	44.6	83.7
Genuine rum..................	28.0	399.0	8.4	2.8	90.6	528.8
Cognac brandy, 10 years old.....	74.5	109.3	16.6	1.6	124.2	326.2
Genuine Scotch whiskey, 8 years old.	48.0	89.7	14.2	4.0	200.0	355.9
Patent spirit for whiskey blending.	8.4	23.8	4.9	0.35	trace	37.4
Sold as whiskey but probably patent spirit.	16.8	8.2	10.0	0.0	0.0	35.0
Brandy mixed with plain spirit...	79.4	32.3	7.35	0.61	49.0	68.7

* These results are in grams per 100 liters of absolute alcohol, and should be divided by two to obtain the corresponding grams per 100 liters of 100° American proof (see page 488).

[1] The Chemistry of Whiskey, *J. Soc. Chem. Ind.*, **1905**, 585.

tionation that the distillate undergoes. There is, of course no such thing in a commercial sense as absolutely pure alcohol, even the highest grades of cologne spirits containing measurable quantities of the secondary products, but the amount present is much less than in the case of such products as pot-still whiskies. A better idea of this variation between various liquors and "silent spirit"[1] may be gained from Table 89, taken from Vasey's "Analysis of Potable Spirits," and further examples are given later.

The specifications of the U. S. Pharmacopœia for medicinal whisky are that the alcohol shall be between 47.0 and 53.0 per cent by volume: the specific gravity between 0.935 and 0.923 at 25°C.; the acids and esters, in terms of grams per 100 liters, between the limits of 48 and 144 for the acids and 17.6 to 70.4 for the esters. The product shall have been aged in charred wood containers for not less than 4 years.

Forms of Adulteration.—From the viewpoint of methods of manufacture, as well as of differences in composition, whiskies may be broadly divided into four classes:[2]

1. Whisky made wholly in a distillery, under government supervision, and usually by processes that produce little or no rectification. If sold as "bottled in bond," this product must be matured for at least 4 years in bonded warehouses and is sold at 100° proof and under a government stamp certifying that these conditions have been met. This whisky is often referred to as "straight" whisky, and, owing to the length of time that it is held to mature, is usually an expensive variety.

2. "Blended" whisky, made by mixing two or more "straight" whiskies in such a manner that the flavor, body, or other qualities may be varied to suit the requirements of the trade. These are also 100° proof and high in price.

3. The third class and by far the largest, is made by mixing a "straight" whisky with silent spirit and water, usually adding some caramel in order to restore the deficiency in color. The terms "blended whisky" and "rectified whisky" are sometimes

[1] *Silent spirit, neutral spirit, velvet spirit, cologne spirit* are all terms used to designate a distilled spirit from which practically all the constituents except ethyl alcohol and water have been separated.

[2] SHEPARD: Assoc. Food and Dairy Depts., 10 *Ann. Proc.*, **1906**, 236.

applied to this product, which is naturally cheaper to manufacture than the whiskies of the preceding classes.

"Blended" whisky is required to contain at least 20 per cent of straight whisky.

4. Still another class of whisky is wholly artificial, being made by adding water and coloring matter, together with various oils and essences to silent spirit. This product is of course not made under government supervision, and it brings the lowest price in the market.

It should be recognized that the above classification is based upon the assumption that the straight or pot-still whisky is a higher grade or more "natural" product than that obtained from the fractionating still. For this point of view there is a strong argument in the fact that straight whisky is the agent which the blender adds to the comparatively flavorless neutral or silent spirit in order to give it the characteristic flavor of whisky.

On the other hand, as stated on page 555, it has been decided officially in both this country and Great Britain that the product of the fractionating still is as much entitled to the name whisky, without qualification, as that obtained by the simpler and older process.

Under the terms of *Food Inspection Decision* 113, all unmixed distilled spirits from grain, colored and flavored with harmless flavor and color, in the customary ways, either by the charred-barrel process, or by the addition of caramel and harmless flavor, if of potable strength and not less than 80° proof, are entitled to the name whisky without qualification. If the proof be less than 80°, *i.e.*, if more water be added, the actual proof must be stated on the label, and this requirement applies as well to blends and compounds of whisky.

Whiskies of the same or different kinds, *i.e.*, straight whisky, rectified whisky, redistilled whisky, and neutral spirits whisky are like substances, and mixtures of such whiskies, with or without harmless color and flavor used for purposes of coloring and flavoring only, are blends under the law and must be so labeled.

It is required also that unmixed potable alcoholic distillates from sources other than grain, if sold as whisky, shall be labeled "Imitation Whisky." Further, that a whisky of a particular flavor, as rye whisky, if made by adding artificial flavor to the

alcoholic distillate from another source, as corn, must be so labeled as to show that it is an imitation.

Under this conception of what constitutes a genuine whisky, the chief form of adulteration would consist in a disagreement of the label with the actual contents of the package, *i.e.*, would be a misbranding. Typical cases might be: (*a*) the refilling of a bonded or "double stamped package" with either an inferior grade of whisky or with alcohol diluted to the proper proof and artificially colored and flavored; (*b*) the "palming off" of one variety of whisky as a more expensive or more desirable kind, such as labeling whisky made of rectified or neutral spirits, colored and flavored, in such a way as to indicate that it was a straight rye or Bourbon whisky; (*c*) incorrect or misleading statements as to the age of the whisky or the materials from which it was made. It should be carefully borne in mind in attempting to show adulteration by chemical tests that whisky is of itself a decidedly variable product, even more so under the terms of its legal definition; that in most cases it will be only the comparatively gross forms of adulteration that can be shown chemically, and that as regards the judging of quality or comparison of flavor more can be ascertained by a properly trained sense of taste than by any chemical analysis.

Methods of Analysis

Statement of Results.—Unless otherwise directed the results obtained should in each case be expressed as grams in 100 liters of *proof spirit*. For example, if 100 liters of whisky of 88° proof contain 15.4 grams of aldehydes this would correspond to 17.5 grams per 100 liters of proof spirit (100° proof).

Specific Gravity.—This is not often needed, but if desired may be determined at $\frac{20°}{20°}$C. by the pyknometer, as described on page 3, taking care to avoid loss of alcohol by undue exposure or heating.

Alcohol.—Determine by distilling 25 cc. as directed on page 486. Report the result as per cent by volume or degrees proof.

Note.—Since whisky ordinarily contains only a slight proportion of extract, the determination of alcohol can be made more quickly and often with sufficient accuracy by means of a suitable hydrometer. The form prescribed by the "Gaugers Manual" of

the U. S. Treasury Department, and accompanied by a complete set of temperature correction tables, is especially convenient. The presence of an appreciable amount of added sweetening would render the results less accurate.

Methyl Alcohol.—See page 502.

Extract.—Evaporate 50 cc. of whisky to dryness on the water bath, best in a platinum dish, dry for 1 hour at 100°C., and weigh.

Notes.—The official method of the Association of Official Agricultural Chemists specifies a sample of 100 cc. and a period of $2\frac{1}{2}$ hours drying, but practically the same results are given by the shorter method.

Care should be taken not to expose the sample at first to the full heat of the bath, since on account of the high alcohol content vigorous boiling and consequent loss of sample may result.

The determination is easily made and gives valuable information if the product has not been quick-aged.

The extract in general will not exceed 0.5 gram per 100 cc., which was the former standard of the U. S. Pharmacopœia[1] but a very liberal one. On the other hand, the often quoted maximum of 0.15 gram per 100 cc. is undoubtedly much too low for modern whisky.

Under normal conditions qualitative tests made on the weighed extract by treating it with 25 cc. of water will give some indication of its having been stored in wooden containers. If this has been the case the residue should not dissolve completely and the filtrate should give a greenish-black color with a few drops of dilute ferric chloride solution (presence of tannin). These tests, however, are not so valuable as formerly because of the use of oak chips in quick-aging processes.

Acids.—Titrate 50 cc. of the sample, diluted to 100 cc. with distilled water, with $0.1N$ alkali, using phenolphthalein as indicator. Calculate the result in terms of acetic acid.

Esters.—Use 100 to 200 cc. of the sample, add 12.5 to 25 cc. of water, and distill 100 to 200 cc., depending on the size of the sample, using a mercury valve to prevent loss of alcohol. Use 50 cc. of the distillate, exactly neutralize the free acid with $0.1N$ alkali and phenolphthalein, add 25 to 50 cc. of $0.1N$ alkali in excess, and either boil for 1 hour with a reflux condenser, cool,

[1] Tenth Decennial Revision.

and titrate with $0.1N$ acid, or allow the solution to stand overnight in a stoppered flask with the excess of alkali, heat with a tube condenser for $\frac{1}{2}$ hour at a temperature below the boiling point, cool, and titrate. Carry out a blank determination at the same time, following exactly the same procedure. Calculate the grams of ethyl acetate corresponding to the number of cubic centimeters of $0.1N$ alkali used to saponify the esters.

Notes.—Saponification is complete by standing overnight in the cold with nearly all samples, but occasionally a low result is obtained, hence the additional precaution of heating for $\frac{1}{2}$ hour before titrating. Hossack, of the Customs Excise Laboratory of Canada,[1] claims that the refluxing with hot alkali can be done in 10 minutes instead of 1 hour and gives figures to demonstrate it.

Because the amount of standard alkali used is small, the alkalinity of the glass flasks is a factor to be taken into consideration. The usual factor derived by standardization of the alkali in the cold should not be relied on. The value obtained from duplicate blank determinations should be used. The determinations and blanks should be carried out in the same kind of flasks, preferably Pyrex, which have been steamed before being used.

In very exact analyses it might be preferable to remove the aldehydes by treatment with metaphenylenediamine, as described below, but the error introduced by their presence is so slight that it may be neglected in practical work.

The mercury valve referred to consists of a small glass U-tube with the bend sealed by a few drops of mercury. This is attached to the receiving flask by a two-holed rubber stopper, through the other opening of which passes the condenser tip. Thus a certain contraction and expansion of the contained air is permitted, while all loss is prevented. If the distillation is conducted slowly and the condenser tip extends some distance into the neck of the receiving flask, there is practically no loss, even without such a valve.

The distillation must be conducted slowly, taking care especially not to overheat the sides of the distilling flask, to avoid decomposition and consequent formation of furfural.

Esters are not produced in the ordinary quick-aging processes, hence are a reliable index to the age of the whisky. The method

[1] *Analyst,* **1935,** 170.

although apparently simple, requires much care and must be followed implicitly in all details to get results of the greatest value.

Aldehydes. *Preparation of Reagents.* ˚*a. Aldehyde-free Alcohol.*—Use the best commercial alcohol obtainable and treat it as described on page 181. Add to the distillate 2 to 3 grams of metaphenylenediamine hydrochloride per liter of alcohol, together with some glass beads, and boil it gently under a reflux condenser for several hours. Distill slowly, allowing the reagent to remain in the alcohol. Reject the first 100 and last 200 cc. of the distillate. The remainder should give no color when tested with the fuchsin-sulphite reagent as described below, and may be preserved for some time in a cool, dark place. The excess of metaphenylenediamine reagent may be filtered off, washed with a little strong alcohol, and dried on a porous tile, when it can be used again with the addition of a little fresh material.

b. Fuchsin-sulphite Reagent.—Dissolve 0.50 gram of pure powdered fuchsin in 500 cc. of warm water, cool, balance on a suitable scale, and pass in sulphur dioxide until the weight has increased by 5 grams. Make up to 1 liter and allow to stand a few hours or until colorless.

Prepare only as much as needed, since the solution retains its strength for only a few days. Keep it in the ice box.

c. Standard Aldehyde Solution.[1]—Grind aldehyde ammonia in a mortar with anhydrous ether and decant the ether. Repeat this operation several times, then dry the purified salt in a current of air and finally in a vacuum over sulphuric acid. Dissolve 1.386 grams of this purified aldehyde ammonia in 50 cc. of 95 per cent alcohol, add 22.7 cc. of normal alcoholic sulphuric acid, then make up to 100 cc. and add 0.8 cc. of alcohol to compensate for the volume of the ammonium sulphate precipitate. Allow this to stand overnight and filter. This stock solution contains 1 gram of acetaldehyde in 100 cc. and will retain its strength if kept cold and dark.

From this stock solution prepare when wanted a standard solution by diluting 2 cc. to 100 cc. with 50 per cent alcohol. This dilute solution should be used the day it is made. (1 cc. = 0.0002 gram of acetaldehyde.)

Determination.—Measure 10 cc. of the distillate obtained in the determination of esters into a 100-cc. Nessler tube, add

[1] VASEY: "Guide to the Analysis of Potable Spirits," p. 31.

25 cc. of the aldehyde-free alcohol, 25 cc. of water, and immerse the tube in a bath of water kept at 15°C. At the same time prepare suitable standards from the standard aldehyde solution, say 0.0, 0.5, 1.0, 2.0, 3.0, and 4.0 cc., add the aldehyde-free alcohol and water as above, and immerse all the tubes in the bath at 15°. Let stand 10 minutes in order to reach the temperature of the bath; then add rapidly to all the tubes 25 cc. of fuchsin-sulphite reagent (which should also be kept in the bath at 15°C.). Allow the tubes to stand in the bath for 15 minutes, then compare the colors. This may be done directly in the tubes or if a more accurate colorimeter, such as the Duboscq (page 27), be employed the solutions may be kept in the bath and transferred quickly to the colorimeter for reading.

Notes.—The treatment of the alcohol with metaphenylenediamine is to remove the last traces of aldehyde which are not taken out by the first treatment, a non-volatile product being formed by condensation of the aldehyde with the two amino groups.

In the preparation of the fuchsin-sulphite reagent the amount of sulphur dioxide added is important, too much making the reagent less sensitive, and too little causing difficulty in getting a satisfactory "blank" with the alcohol itself. If difficulty should be experienced in preparing an alcohol which is absolutely aldehyde-free, it is possible to increase slightly the sulphur dioxide, say up to 6 grams per liter, without seriously interfering with the delicacy of the reaction.

The treatment of the aldehyde ammonia with ether is to remove the polymerized aldehyde resin which is present through partial decomposition. The purified product should be perfectly white and readily soluble in alcohol. This method of preparing a standard aldehyde solution is preferable to weighing out the aldehyde directly, since the pure substance polymerizes so readily, thus changing in strength.

It is important in this determination that the temperature should be kept at 15° and that all the samples should be at the same temperature, since the color deepens greatly with a slight rise in temperature. It should be noted also that the color developed in the reaction is not directly proportional to the amount of aldehyde present; hence the reading of the sample should not be far different from that of the standard with which it is compared. If in any particular case this should be imprac-

ticable, the reading may be corrected by a table worked out by Tolman.[1]

Furfural, although an aldehyde, is not included in the determination, on account of the very faint color it gives with the fuchsin-sulphite reagent as compared with acetic aldehyde.

The aldehyde content increases during storage, owing to concentration of the whisky, but if calculated back to the original volume there is actually a loss.

Furfural. *Standard Furfural Solution.*—Weigh 1 gram of redistilled furfural and dissolve it in 100 cc. of 95 per cent alcohol. This strong solution will keep (best cool and in the dark). Prepare the dilute solution for making the standards by diluting 1 cc. of this strong solution to 100 cc. with 50 per cent, by volume, alcohol. Each cubic centimeter of this solution contains 0.0001 gram of furfural.

Determination.—Measure 10 or 20 cc. of the distillate obtained in the determination of esters into a 100-cc. Nessler tube, add 25 cc. of alcohol free from furfural,[2] 25 cc. of water, and immerse the tube in a bath of water kept at 15°C. At the same time prepare suitable standards from the standard furfural solution, say 0.0, 0.5, 1.0, 2.0, 3.0, and 4.0 cc., add the furfural-free alcohol and water as above, and immerse all the tubes in the bath at 15°. Let stand for 10 minutes, in order to reach the temperature of the bath, add to the tubes 2 cc. of colorless (redistilled) anilin, and then 0.5 cc. of hydrochloric acid (sp. gr. 1.125). Allow the tubes to remain in the bath for 15 minutes and then compare the colors as in the case of the aldehyde determination (page 567).

Notes.—The same reaction has been used as a qualitative test for furfural on pages 261 and 334, the only difference being that acetic acid was used instead of hydrochloric, and no precautions were taken as regards the temperature. Acetic acid can be used just as well but in some instances has been found to contain traces of furfural or some impurity that reacts similarly. For quantitative tests the control of the temperature is highly important since it greatly affects the depth of color. At the low temperature chosen the reaction is perhaps less delicate but is more readily controlled.

[1] *J. Am. Chem. Soc.*, **1906**, 1627.

[2] This may be prepared by treatment of commercial alcohol, as described on p. 181.

Authorities differ as to whether the depth of color is directly proportional to the amount of furfural present; hence the strength of the sample should not be far different from that of the standard with which it is compared.

The furfural is easier to check than the aldehydes because it is less sensitive to temperature changes during the analysis. The main source of the furfural is the charred barrel. Uncharred white-oak wood does not contain furfural. Practically all is taken up during the first 6 months contact with the charred barrel.

In order to avoid the possibility of forming furfural by decomposition during the distillation, Schidrowitz[1] has proposed to determine the furfural directly in the sample without distilling, removing the bulk of the color with lead acetate. This, however, works satisfactorily only with light-colored samples, so that the test is best made on the carefully prepared distillate.

Higher Alcohols (Fusel Oil). *Discussion.*—The term "fusel oil" comprises the flavor-producing substances that are inherent in the freshly distilled liquor, *i.e.*, chiefly the higher alcohols and their esters. The esters, being decomposed by saponification before the higher alcohols are determined, are necessarily included. As previously stated, the principal constituents of this group are normal and isoamyl alcohols, isobutyl alcohol, and smaller quantities of normal and isopropyl alcohols, with the corresponding esters.

Many methods have been proposed for this determination, but it may be said frankly at the outset that there is no entirely satisfactory process. A particular method may give comparable values to an experienced analyst, but different procedures will give very different results, not only because of fundamental differences in the reactions involved but also because the fusel oil itself is of variable composition as found in different classes of potable spirits.

Of the numerous methods two have been selected for detailed discussion because they are typical of the classes most widely used, one depending upon oxidation of the alcohols to definite products, the other a quicker and simpler colorimetric method.

a. Allen-Marquardt Method. Principle.—This method involves extraction of the higher alcohols by carbon tetrachloride, in which

[1] *J. Soc. Chem. Ind.*, **1902**, 815.

they are more readily soluble than is ethyl alcohol, washing of the carbon tetrachloride free from traces of ethyl alcohol, oxidation of the extracted alcohols of the fusel oil to the corresponding acids by potassium bichromate, and distillation and titration of the acids formed.

Procedure.—Add to 50 cc. of whisky 50 cc. of water, then 20 cc. of approximately $0.5N$ sodium hydroxide and saponify the mixture by boiling for 1 hour under a reflux condenser. Connect the flask with a distilling apparatus, distill 90 cc., add 25 cc. of water, and continue the distillation until an additional 25 cc. is collected.

If aldehydes are present in excess of 15 grams per 100 liters, add to the distillate 0.5 gram of metaphenylenediamine hydrochloride, boil under a reflux condenser for 1 hour, distill 100 cc., add 25 cc. of water, and continue the distillation until an additional 25 cc. is collected. Approximately saturate the distillate with finely ground sodium chloride and add a saturated solution of sodium chloride until the specific gravity is 1.10, as determined by a small hydrometer or the Westphal balance.

Extract this salt solution four times with carbon tetrachloride, purified as described in the Notes below, using 40, 30, 20, and 10 cc., respectively, and wash the carbon tetrachloride three times with 50-cc. portions of a saturated solution of sodium chloride and twice with a saturated solution of sodium sulphate. Note the precautions given for quantitative extractions on page 107. Then transfer the carbon tetrachloride to a flask containing 5 cc. of concentrated sulphuric acid, 45 cc. of water, and 5 grams of potassium bichromate, and boil for 8 hours under a tall reflux condenser.

Add 100 cc. of water and distill until only about 50 cc. remains; add 50 cc. of water and distill until 35 to 50 cc. is left. Use extreme care to prevent the oxidizing mixture from burning and baking on the sides of the flask. The distillate should be water white; if colored, discard it and repeat the determination. Titrate the distillate with $0.1N$ sodium hydroxide, using phenolphthalein as indicator. Calculate the final titration to amyl alcohol (1 equivalent of alkali = 1 mol of the alcohol).

Rubber stoppers can be used in the saponification and first distillation, but corks covered with tin foil must be used in the oxidation and second distillation. Corks and tin foil must be

renewed frequently. Standard taper glass joints are an improvement over corks. Make a blank determination on the carbon tetrachloride, using 100 cc., and beginning at the point in the procedure where the carbon tetrachloride is washed with saturated sodium chloride solution.

Notes.—It is essential that the carbon tetrachloride used be of the highest purity, and it will generally be found necessary to purify the reagent. This may be done by boiling with potassium bichromate and sulphuric acid, and distilling over barium carbonate, or more conveniently as follows:[1]

Mix the impure carbon tetrachloride with one-tenth its volume of strong sulphuric acid. Shake the mixture thoroughly at frequent intervals and allow it to stand overnight. Then run water through the mixture continuously, by means of a glass tube inserted to the bottom of the bottle and connected with the water tap, until thoroughly washed free from acid and impurities. Draw off the upper layer of water by means of a siphon, the last portions being removed as far as possible with a pipette. Add an excess of soda solution and distill the carbon tetrachloride from it.

The endeavor should be made during the entire Allen-Marquardt process to maintain uniform conditions so far as possible. The shaking should be with a long swinging motion, rather than quick and violent, and continued for 2 minutes each time. The solutions of sodium chloride and sodium sulphate should be perfectly saturated, best kept standing over some of the finely powdered salt. The temperature should be kept uniform and as low as convenient, since it has been found that a low temperature tends toward more efficient extraction. The boiling of the carbon tetrachloride with the oxidizing solution should be slow and regular, and a high condenser should be used to ensure the complete condensation of all the products.

The saponification with sodium hydroxide before distilling is for the purpose of holding back the esters and furfural, which would otherwise pass into the distillate. The washing of the carbon tetrachloride with saturated sodium chloride is to remove the small quantity of ethyl alcohol which is extracted along with the amyl alcohol. This must in turn be removed by thorough washing with sodium sulphate, since any chloride that remains

[1] BRECKLER: U. S. Dept. Agr., *Bur. Chem. Bull.* **122**, p. 209.

will cause trouble through being oxidized to chlorine by the bichromate mixture and hence interfering with the titration by bleaching the indicator. A few drops of dilute sodium thiosulphate added just before the titration will be found an advantage. More than two washings with the sodium sulphate cannot be made without danger of removing some of the higher alcohols. Rubber stoppers should not be used during the oxidation and final distillation, on account of the possibility of action by the oxidizing mixture; corks, on the other hand, are liable to absorb amyl alcohol and valeric acid, but can be used if carefully covered with tin foil.

The Allen-Marquardt is the method most widely used in English-speaking countries and constitutes the official method of the Association of Official Agricultural Chemists. If carried out with strict regard to details it gives excellent checks, and a great deal of data has been accumulated and published based upon it.

It is, however, lengthy and troublesome. It is not scientifically accurate, since all the higher alcohols are expressed in terms of amyl alcohol. Isopropyl alcohol, if present, is not taken care of because it is oxidized to acetone; secondary isobutyl alcohol, if oxidized to ketone, is missed, and if oxidized further, will give two molecules of acetic acid instead of one, causing high results. It gives only about 65 to 75 per cent recovery of normal and isoamyl alcohols and very much less of the butyl and propyl alcohols.

In spite of these many drawbacks it must be regarded at the present time as the standard method. It should be said definitely, however, that unless the analyst is willing to spend considerable time in becoming proficient with the method it is better to use the colorimetric procedure or to omit the fusel-oil determination entirely and be content with the information to be obtained from the other determinations, because the Allen-Marquardt method is of no use unless carried out with the greatest care.

b. Colorimetric Method. Discussion.—A color method largely used in France is based on the color given by isobutyl alcohol with strong sulphuric acid.[1] The amyl alcohols, however, give but little color with sulphuric acid under these conditions, and more general colorimetric methods are based on the Komarowsky

[1] GIRARD and CUNIASSE: "L'Analyse des Alcools," 1899.

reaction.[1]   In this, colored products are formed through the interaction of the higher alcohols with cyclic aldehydes in the presence of sulphuric acid.   This color reaction, however, is not peculiar to the higher alcohols but includes, among other constituents of whisky, aldehydes, acetals, and ketones.   These interfering substances must be removed before the colorimetric test can be applied to liquors.   This has been done and the color reaction applied to whisky by Penniman, Smith and Lawshe,[2] who describe a simple and accurate procedure of this kind.

Principle.—Interfering substances are removed and the esters saponified by alkaline silver oxide.   After distillation, the higher alcohols are converted by sulphuric acid into unsaturated hydrocarbons which are then combined with salicylaldehyde or vanillin to form colored solutions.   These are compared with the colors given by known amounts of natural or synthetic fusel oil.

Procedure.   a. Preparation of Sample.—Place 25 cc. of the whisky in a 500-cc. round-bottomed flask.   Add 0.5 gram of silver sulphate, 1 cc. of sulphuric acid $(1 + 1)$ and 90 cc. of water. Boil gently under a reflux condenser for 15 minutes.   Make the solution alkaline with 5 cc. of sodium hydroxide solution $(1 + 1)$ and reflux again for 30 minutes.   Bumping can be prevented by the addition of a little granulated zinc, and if foaming occurs this can be helped by adding 15 grams of sodium chloride.   After saponifying, distill 75 cc., which will contain all of the higher alcohols present in the original sample.

b. The Color Reaction.—Measure accurately with a pipette 2.00 cc. of the distillate obtained above into a 125-cc. flat-bottomed, long-necked flask (Florence flask).   Add 20.0 cc. of concentrated sulphuric acid (only 10 cc. if the vanillin reagent is to be used), swirling the flask in a bath of cold water during the addition.   Then add (measured carefully) 2.00 cc. of the reagent,[3] again swirling the flask in a cold bath.

Prepare a similar flask containing 2.00 cc. of a standard fusel oil solution (see Notes), acid, and reagent.

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[1] *Chem.-Ztg.*, **1903**, 807, 1086.

[2] *Ind. Eng. Chem., Anal. Ed.*, **1937**, 91.

[3] If salicylaldehyde is used the reagent solution contains 10 mg. per cc. of 95 per cent alcohol; if vanillin, the strength is 17.5 mg. per cc. of 95 per cent alcohol.   In either case the ordinary C.P. grade of chemicals will be satisfactory.

Place the flasks simultaneously in a bath of vigorously boiling water. After 20 minutes transfer the flasks to the cold bath. When cool, add 25 cc. of sulphuric acid $(1 + 1)$ and mix thoroughly by swirling. Compare the colors in a Duboscq or similar colorimeter.

Notes.—The success of the colorimetric method depends upon the use of accurate and reliable color standards. The most satisfactory standard is a solution containing a weighed amount of actual fusel oil of the type contained in the sample. Where a standard fusel oil is not available a satisfactory working standard can be prepared by mixing isoamyl (boiling point 132°) and isobutyl alcohols in the ratio of 4 to 1. In using the synthetic standard the colorimeter reading should be multiplied by 0.95 if using salicylaldehyde, and by 0.90 if using vanillin, since the synthetic standard gives a slightly weaker color than the fusel oil.

The percentage of ethyl alcohol in the standard should be approximately the same as in the prepared sample, or 15 per cent by volume for whisky originally 90 to 100° proof. A convenient fusel-oil concentration for the standard is 0.1 gram per 100 cc.

With increasing concentration of fusel oil there is a slight shift in color tone from red toward purple. This makes it best to have the sample and standard of approximately the same concentration.

The addition of salt in the removal of the aldehydes converts the silver oxide partly to chloride but has little effect on the efficiency of the reaction. The dilution to one-third of the original concentration (25 cc. to 75 cc.) is made necessary by the extreme sensitivity of the color reaction.

In carrying out the color reaction numerous factors must be carefully controlled in order to give a clear solution with a color intensity suitable for examination in the colorimeter. Further, the reaction is not allowed to go to completion, but is stopped by chilling and diluting. It is therefore necessary to run sample and standard side by side under exactly similar conditions.

For a further discussion of the fusel oil determination and a critical study of the methods the student is referred to the following papers in addition to those cited:

Schidrowitz and Kaye, *Analyst*, **1905**, 190; **1906**, 181.
Mann, *J. Soc. Chem. Ind.*, **1906**, 1125.
Tolman and Hillyer, U. S. Dept. Agr., *Bur. Chem. Bull.* **122**, p. 206.
Dudley, *J. Am. Chem. Soc.*, **1908**, 127.

Color.—The total color is customarily reported in terms of the standard colored glasses of the American Society of Brewing Chemists, Series No. 52 (the so-called "brewers' scale") as read in a Lovibond tintometer. Beyer[1] has suggested the use of an abridged spectrophotometer as giving closer readings.

While the *degree* of color present in whisky has no special analytical significance, the *character* of the color is of great importance in judging the nature of the sample, since the color of straight whisky is originally derived entirely from the wooden package in which it is stored, while that of compounds or imitations usually consists wholly or in part of caramel. The difference is best shown through certain considerations of solubility.

a. Color Insoluble in Water.—Evaporate 50 cc. of the whisky just to dryness on the water bath, take up the residue in 15 cc. of cold distilled water, and filter. Wash the paper thoroughly with small portions of water until the filtrate amounts to nearly 25 cc., and add enough alcohol (25 cc. of absolute or 26.3 cc. of 95 per cent) to make 50 cc. (the original volume). Mix and compare the color of this solution in a colorimeter with that of the untreated whisky and calculate the percentage of color remaining, which, subtracted from 100, gives the percentage of color insoluble in water.

Note.—Caramel, the artificial color most commonly used in whisky, is of course readily soluble in water, while *flavescein*, the coloring matter taken up by straight whisky from the oak wood in which it is stored, is much less soluble. A genuine whisky will seldom show less than 70 per cent of color insoluble in water.

b. Color Insoluble in Amyl Alcohol.—Evaporate 50 cc. of the whisky just to dryness in a porcelain dish on the water bath. Add 26.3 cc. of 95 per cent alcohol to dissolve the residue and transfer to a 50-cc. flask. Wash out the dish and make up to the mark with water. Place 25 cc. of the 50 per cent alcoholic solution in a separatory funnel, add 20 cc. of freshly shaken Marsh reagent, and proceed as described on page 460 in detecting caramel in vanilla extract. Calculate the percentage of color not soluble in amyl alcohol.

[1] *J. Assoc. Off. Agr. Chem.*, **1939**, 156.

Notes.—The test depends upon the relative solubility of coloring matters in ethyl alcohol, amyl alcohol and water. The addition of amyl alcohol, when in sufficient quantity, to a mixture of 50 parts of ethyl alcohol and 50 parts of water will cause a separation of the liquids into two layers, the lower layer being largely water and the upper one a mixture of ethyl alcohol, amyl alcohol, and some water. As a result of this division, water-soluble coloring matter can be separated from alcohol-soluble coloring matter; *i.e.*, caramel can be separated from the natural coloring matter of whisky.

In a straight American whisky about 90 per cent. of the coloring matter is soluble in the amyl alcohol-ethyl alcohol layer, as compared with approximately 15 per cent. in the case of imitation whiskies. The method gives a sharper differentiation between caramel and the coloring matter taken from wood in the natural aging process than even the water-insoluble method.

Artificial Color. *a. Amyl Alcohol Test.*—Shake 5 cc. of the whisky in a test tube with two or three times its volume of amyl alcohol. In the presence of artificial color a pronounced color will usually appear in the lower layer.

Note.—The test shows well the presence of artificial color but does not distinguish between coal-tar dyes and caramel, and may show a positive result with aged whiskies even when no artificial color has been added. If a positive result is obtained the Marsh test, *b*, should be tried.

b. Marsh Test.—To 10 cc. of the sample in a 20-cc. test tube, add sufficient Marsh reagent (page 460) nearly to fill the tube, and shake several times. Allow the layers to separate. Color in the lower layer indicates that the sample has been colored with caramel, a coal-tar dye, or with extractive material from uncharred white oak chips.

Notes.—A genuine naturally aged whisky should give a negative or practically negative test with the Marsh reagent. A positive test may be due to caramel, to a few coal-tar colors, or to uncharred or partially charred (toasted) white-oak chips. Practically all coal-tar colors that give a satisfactory brown color with whisky show positively in the amyl alcohol test, *a*, but usually show almost no color in the lower layer in the Marsh test.

A negative test does not mean necessarily the absence of artificial color (some coal-tar colors give a negative result) but usually does. Through the use of various quick-aging processes

the problem has been complicated somewhat, the reddish-brown color extracted from uncharred white-oak chips giving a positive Marsh test. This form of color, easily extracted by proof alcohol, is different from the color produced by slow-aging in charred barrels or by ordinary quick-aging in the presence of toasted or charred chips. If then, the Marsh test is positive, the following modified procedure[1] should be applied to confirm the presence of caramel.

c. Modified Marsh Test (Zinc Acetate Test).—Place 25 cc. of spirits in a 150-cc. beaker marked to show volumes of 13 cc. and of 25 cc., add 0.5 cc. of glacial acetic acid, 0.75 gram of zinc acetate crystals, and mix. When nearly dissolved, boil down rapidly over a flame to the 13-cc. mark, stirring frequently to prevent bumping or spattering. If the liquid should inadvertently go below the 13-cc. mark, fill to that mark with water and set aside to cool. When cooled to room temperature, fill to the 25-cc. mark with 95 per cent alcohol, mix, and allow to stand 2 to 3 minutes. Mix again and filter through a double filter (folded or S. S. 589 white ribbon). Mix the filtrate and transfer 6 cc. to a 6-in. test tube; add 12 cc. of Marsh reagent and mix thoroughly until the voluminous white precipitate that forms when the liquids first mix goes back into solution. Allow to stand until the layers separate, then pour off 4 cc. of the upper layer into a graduated cylinder, and in its place in the test tube pour 4 cc. of ethyl acetate, mix, and allow to stand until the layers separate. A dark-brown color in the lower layer indicates that caramel is present. If the lower layer has a reddish shade, coal-tar colors may be present. Confirm the presence of coal-tar color by transferring some of the remaining filtrate to a porcelain dish and adding a few drops of hydrochloric acid. If coal-tar colors are present, the solution may become red. To confirm the presence of coal-tar color add stannous chloride solution, which will remove the coal-tar color. Use the clear light of an open window as a background for examining the colors obtained in this test.

d. Ferric Alum-Sodium Acetate Test.—Another test for whisky that is suspected of being colored with uncharred chips or similar coloring matter is as follows:

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[1] MALLORY and VALAER: *Ind. Eng. Chem., Anal. Ed.*, **1934**, 474; *J. Assoc. Off. Agr. Chem.*, **1935**, 75.

To about 10 cc. of the whisky add 2 cc. of ferric ammonium sulphate solution (1 per cent in water), followed by 5 grams of powdered sodium acetate. Mix, allow to stand for 10 minutes, and filter.

Notes.—If the color is due to uncharred white-oak chips, the solution becomes an intense blue-black on the addition of the ferric alum, but the filtrate is without color. Sometimes the filtrate is slightly bluish, becoming colorless on being warmed gently and poured through the filter several times.

If caramel is present, warming and refiltering does not affect the color. The same is true if the whisky was colored by quick-aging with charred or toasted oak chips, or by natural aging in wood over a period of years as with "bottled in bond" whisky.

Another qualitative test of value for showing quick-aging treatment is to add to 5 cc. of whisky approximately 1 gram of calcium chloride, shake continuously until dissolved, cool, and then shake with Marsh's reagent as in *b*. After shaking let the mixture stand. A heavy brown ring that forms at the junction of the two layers is given with whiskies that have been treated with either charred or uncharred chips.

Interpretation of Results

It will be evident from what has been said already that the chemical analysis is best restricted to determining certain comparatively gross forms of adulteration.

The addition or substitution of neutral spirits, suitably colored and flavored, for straight whisky is a typical instance of this kind. It is a form of adulteration frequently practiced, and will serve as a suitable illustration of the interpretation of the chemical analysis.

Authentic Analyses of Whisky.—The most extended as well as instructive series of analyses of authentic samples of whisky are undoubtedly those made under the direction of Crampton and Tolman[1] in a study of the changes that occur when whisky is aged in wood and a similar more recent study by Valaer and Frazier,[2] from which papers the material given here is freely taken. In the first of these studies, 31 barrels of whisky were kept in bonded warehouses for the aging period of 8 years, a

[1] *J. Am. Chem. Soc.*, **1908**, 98.
[2] *Ind. Eng. Chem.*, **1936**, 92.

sample being removed each year and kept in glass until all were analyzed at the end of the bonded period. The maximum, minimum, and average results obtained for various periods are shown in Table 90. To eliminate some abnormal results, the next to the lowest figures are also included in most instances. The color is reported on the "brewer's scale" of the Lovibond tintometer on all the samples, hence gives comparative data for depth of color. The other determinations were made according to the methods described on pages 563 to 578. No caramel was found in any sample.

The results show a gradual increase in the constituents determined during the 8 years. This is due to chemical changes and also, especially after the fourth year, to concentration, the water of the spirit, and to a lesser degree the alcohol, evaporating through the wood to a much greater extent than do the other constituents. The charring of the barrel has a pronounced effect on the body, color, and flavor of the whisky, samples that had been kept for 8 years in uncharred barrels having only as much of these characteristics as were produced by aging for 2 years in the charred package.

For judging the character of a sample in the light of these analyses certain general considerations should be pointed out. The maximum and minimum figures are given only to show the range of values that may be expected and are not to be used directly in judging a whisky, since they do not coincide, except in one instance pointed out later. The fact that a given sample falls between the maximum and minimum figures given for a particular age is in itself no definite criterion of its genuine character. The average figures are of more value, but it is chiefly upon certain relationships that reliance must be placed.

Rye and Bourbon whiskies show certain characteristic differences, a typical one being the rate of increase in proof. This is accounted for by the fact that the rye whisky is usually aged in heated warehouses, where the changes and evaporation taking place are aided by the higher temperature. In every other way the rye whiskies show this increased activity, containing more solids, color, acids, esters, etc.

The solids and color form the one exception noted above. Since with straight whisky these are both derived entirely from the package, it is to be expected that there should be a very close

TABLE 90.—COMPOSITION OF AMERICAN WHISKY AT VARYING AGES

Sample		Proof	Color	Grams per 100 liters of proof spirit					
				Extract	Acids	Esters	Aldehydes	Furfural	Fusel oil
Rye whisky									
New	Average...	101.2	0.0	13.3	12.0	16.3	5.4	1.0	90.4
	Maximum	102.0	0.0	30.0	72.0	21.8	15.0	1.9	161.8
	Minimum.	100.0	0.0	5.0	4.4	4.3	0.7	trace	{ 61.8 / 43.7 }
One year old	Average...	102.5	8.8	119.7	46.6	37.0	7.0	1.8	111.5
	Maximum	104.0	13.8	171.0	60.5	64.8	15.5	3.3	194.0
	Minimum.	101.0	{ 7.2 / 6.6 }	{ 93.0 / 92.0 }	{ 31.1 / 5.8 }	{ 6.8 / 6.8 }	2.8	0.4	{ 80.4 / 66.4 }
Two years old	Average...	104.9	11.6	144.7	51.9	54.0	10.5	2.2	112.4
	Maximum	109.0	16.7	199.0	75.6	75.1	18.7	5.7	214.0
	Minimum.	100.0	{ 8.8 / 8.6 }	{ 121.0 / 94.0 }	{ 44.3 / 11.0 }	{ 41.5 / 31.2 }	5.4	0.7	{ 83.4 / 82.2 }
Four years old	Average...	111.2	14.0	185.0	65.9	69.3	13.9	2.8	125.1
	Maximum	118.0	18.9	238.0	83.8	89.1	22.1	6.7	203.5
	Minimum.	105.0	{ 11.6 / 11.3 }	{ 156.0 / 153.0 }	{ 58.6 / 17.3 }	{ 57.7 / 36.3 }	6.4	0.7	{ 83.8 / 67.8 }
Six years old	Average...	118.0	17.0	223.1	72.4	80.4	14.6	3.3	145.5
	Maximum	132.0	21.2	284.0	95.8	109.0	22.3	8.3	245.3
	Minimum.	110.0	{ 13.7 / 12.4 }	{ 193.0 / 176.0 }	{ 67.1 / 24.3 }	{ 64.0 / 39.1 }	7.3	0.7	{ 99.2 / 80.0 }
Eight years old	Average...	123.8	18.6	256.0	82.9	89.1	16.0	3.4	154.2
	Maximum	132.0	24.2	229.0	112.0	126.6	26.5	9.2	280.3
	Minimum.	112.0	{ 13.8 / 13.7 }	{ 214.0 / 200.0 }	{ 73.7 / 31.7 }	{ 68.4 / 40.9 }	7.9	0.8	{ 109.0 / 107.1 }
Bourbon whisky									
New	Average...	101.0	0.0	26.5	10.0	17.4	3.2	0.7	100.9
	Maximum	104.0	0.0	161.0	29.1	53.2	7.9	2.0	171.3
	Minimum.	100.0	0.0	4.0	1.2	13.0	1.0	trace	{ 71.3 / 42.0 }
One year old	Average...	101.8	7.1	99.6	41.1	28.6	5.8	1.6	110.1
	Maximum	103.0	10.9	193.0	55.3	55.9	8.6	7.9	173.4
	Minimum.	100.0	{ 5.4 / 4.6 }	{ 61.0 / 54.0 }	{ 24.7 / 10.4 }	{ 17.2 / 10.4 }	2.7	trace	{ 58.0 / 42.8 }
Two years old	Average...	102.2	8.6	126.8	45.6	40.0	8.4	1.6	110.1
	Maximum	104.0	11.8	214.0	61.7	59.8	12.0	9.1	197.1
	Minimum.	100.0	{ 6.9 / 5.7 }	{ 81.0 / 78.0 }	{ 25.5 / 23.5 }	{ 24.4 / 11.2 }	5.9	0.4	{ 86.2 / 42.8 }
Four years old	Average...	104.3	10.8	151.9	58.4	53.5	11.0	1.9	123.9
	Maximum	108.0	14.8	249.0	73.0	80.6	22.0	9.6	237.1
	Minimum.	100.0	{ 8.6 / 7.4 }	{ 101.0 / 92.0 }	{ 40.0 / 40.0 }	{ 28.2 / 13.8 }	6.9	0.8	{ 95.0 / 43.5 }
Six years old	Average...	107.9	13.1	185.1	67.1	64.0	11.9	1.8	135.3
	Maximum	116.0	17.5	287.0	81.0	83.9	23.3	9.5	{ 240.0
	Minimum.	102.0	{ 12.0 / 9.8 }	{ 132.0 / 127.0 }	{ 53.6 / 45.0 }	{ 36.4 / 17.9 }	7.7	0.9	98.1 }
Eight years old	Average...	111.1	14.2	210.3	76.4	65.6	12.9	2.1	143.5
	Maximum	124.0	20.9	326.0	91.4	93.6	28.8	10.0	241.8
	Minimum.	102.0	{ 12.3 / 10.5 }	{ 152.0 / 141.0 }	{ 64.1 / 53.7 }	{ 37.7 / 22.1 }	8.7	1.0	{ 110.0 / 47.6 }

relationship between them. As a matter of fact, they show such similarity in rate of increase that it is possible to calculate quite closely from one what the other should be, a relationship that is of the greatest value in detecting artificial color in whisky and deciding whether the solids are normal.

The relation between the acids and esters is important in distinguishing matured samples from young or imitation spirits. In the aging process the acids are formed at first more rapidly than the esters, but later the esters form more rapidly, so that the two reach an equilibrium in about 4 years. In the new whisky the acids are distinctly lower than the esters, but at the end of the first year they are nearly the same and even higher, a relationship that gradually draws nearer, until equilibrium is reached at the fourth year. In a given sample, then, a serious discrepancy in this respect would indicate that the whisky was either not genuine or not properly matured.

In general, it may be said that a high color, high solids, and high concentration should be accompanied by high acids and esters, and, conversely, low color and solids go with low acids and esters. Distinct discrepancies in this respect would create a suspicion that the sample was not a genuine straight whisky.

In the study made by Valaer and Frazier the samples were all straight whiskies distilled at or below 160° proof. A variety of products was represented: two sweet-mash ryes, two sour-mash ryes, two sweet-mash Bourbons and five sour-mash Bourbons. Four of the samples had received quick-aging treatment.

The figures obtained (Table 91) have a slightly different significance from those just discussed owing to the extensive practice of quick-aging whiskey before it goes on the market, using higher temperatures and oak chips or barrels that have been charred to varying degrees. This means that the acids are now less important than the esters for indicating age. The esters show little change by the quick-aging process, which mostly increases acids, solids, color, and furfural if heat and charred chips or barrels have been used. Uncharred chips produce high solids or acid content but less color and ash. Treatment of either kind has but little effect on fusel oil and aldehydes. Almost any depth of color can be obtained, but the experienced analyst can distinguish it from the color of natural aging by the altered shade when examined in the Lovibond tintometer.

Although the results reported by Valaer and Frazier are in general similar to those of Crampton and Tolman, some notable differences were found. The general character of the changes produced in the aging is shown in the graph, Fig. 82, taken from their work. The largest increase of acids, esters, solids and color takes place in the first 6 months of storage. The analyses showed no fixed relationship between acids and esters as was observed by the previous workers.

Calculated to the original volumes, there is an actual as well as apparent gain in acids over the 4-year period. The actual
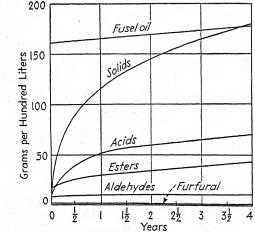
FIG. 82.—Changes in composition of aged whisky.

acid gains varied from 24.9 to 56 grams per 100 liters (average = 40.1). The same is true of the esters, the gain being 7.4 to 21.3 grams per 100 liters (average = 15.5). There is an actual loss of fusel oil of from 6.9 to 58.4 grams per 100 liters (average = 28.6).

Crampton and Tolman assumed that no change takes place during storage in glass, but the later series of tests showed that slight changes do occur, but of course more slowly than in wood. Most notable are the decrease in acids (due possibly to alkali taken from the glass), an increase in color, and a marked improvement in flavor.

Examples of commercial "bottled in bond" rye and Bourbon American whiskies are given in Table 92, and analyses of genuine

TABLE 91.—FURTHER DATA ON AMERICAN WHISKY AT VARYING AGES

Sample		Proof	Color	Grams per 100 liters of proof spirit					
				Ex-tract	Acids	Esters	Alde-hydes	Fur-fural*	Fusel oil
New	Average	**101.2**	....	**10.5**	**7.7‡**	**17.0**	**7.6**	...	**161.1**
	Maximum	102.6	....	20.1	9.6	21.5‡	20.8	...	230.7
	Minimum	100.0	....	2.9	5.3	13.7	2.4	...	78.7
6 months old	Average	**101.3**	**7.1**	**92.5**	**40.3**	**26.5**	**9.6**	**1.7**	**166.8**
	Maximum	102.9	9.9	121.9	52.4	32.8	22.7	2.2	244.3
	Minimum	100.0	4.4	61.6	31.7	18.2	3.9	0.6	87.0
1 year old	Average	**101.9**	**8.6**	**114.4**	**50.1**	**29.9**	**10.2**	**1.9**	**166.5**
	Maximum	103.3	12.2	135.1	53.4†	35.3	20.5	2.2	245.4
	Minimum	100.5	6.3	89.8	38.5†	21.9	4.3	0.6	92.0
1½ years old	Average	**102.8**	**9.8**	**131.3**	**55.9**	**32.4**	**10.4**	**1.9**	**167.0**
	Maximum	104.0	13.6	160.7	62.8	38.2	21.8	2.2	240.4
	Minimum	100.7	7.2	98.4	44.7	22.4	4.7	0.8	96.7
2 years old	Average	**103.8**	**10.8**	**143.0**	**59.7**	**34.7**	**11.0**	**1.9**	**168.3**
	Maximum	105.3	14.5	166.7	65.6	38.7	24.5	2.2	232.1
	Minimum	101.2	7.7	113.7	48.2	25.2	4.6	0.8	97.6‡
2½ years old	Average	**104.8**	**11.9**	**155.8**	**62.8**	**37.0**	**11.0**	**2.1**	**168.6**
	Maximum	106.1	15.6	183.1	72.6	43.1‡	22.7	2.4	237.7
	Minimum	101.9	8.6	125.0	51.4	28.3	4.6	0.8	96.6
3 years old	Average	**105.6**	**12.3**	**163.0**	**65.2**	**38.9**	**11.1**	**2.1**	**172.0**
	Maximum	107.3	15.7	197.9	73.6	43.9§	22.6	2.7	249.8
	Minimum	102.4	9.4	129.8	54.1	28.5	4.6	0.8	98.4‡
3½ years old	Average	**106.6**	**13.1**	**172.8**	**67.9**	**40.0**	**11.2**	**2.1**	**171.2**
	Maximum	109.0	16.1	207.4	74.8	46.2	22.1	2.9	241.3
	Minimum	102.8	9.8	132.5	58.9	29.1	5.2	0.8	95.6
4 years old	Average	**107.7**	**14.1**	**178.7**	**70.6**	**45.0**	**11.6**	**2.2**	**178.5**
	Maximum	110.7	17.3	213.8	78.6	48.8‡	21.7	3.0	260.8
	Minimum	104.0	10.2	141.8	59.8†	37.6	6.0	0.8	96.0

* In the maximum furfural the quick-aged samples and one abnormal one were omitted.
† Does not include quick-aged samples.
‡ Highest or lowest were omitted as slightly abnormal and next taken instead.
§ Highest and next were omitted as slightly abnormal.

Scotch and Irish whiskies, taken from various authorities, in Table 93.

It should not be understood, from the fact that the analyses of "genuine" whisky which are given are all analyses of pot-still spirit, that nothing else is properly entitled to be called whisky. This has been previously pointed out on page 555. The analyses

have been purposely selected because the substitution of straight whisky by neutral spirits is the particular form of adulteration chosen for study.

Analyses of "neutral" or "silent" spirits or so-called "blending goods," typical of the distillates from rectifying stills which

TABLE 92.—ANALYSES OF "BOTTLED IN BOND" AMERICAN WHISKIES

Source	Age	Grams in 100 liters of proof spirit					
		Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
Rye........	8 years	189.8	79.2	81.8	17.5	3.0	84.9
Rye........	7 years	212.0	79.5	94.0	22.5	5.0	119.5
Rye........	7 years	280.0	93.1	86.1	10.3	4.5	162.0
Rye........	8 years	222.0	91.7	87.6	11.9	4.7	116.0
Rye........	4 years	174.0	75.7	71.0	9.7	1.1	194.0
Bourbon....	8 years	181.5	59.1	60.7	17.5	3.2	102.6
Bourbon....	4 years	148.5	54.9	55.9	15.0	2.6	152.0
Bourbon....	5 years	129.3	53.7	49.3	9.5	0.8	141.7
Bourbon....	7 years	220.0	88.2	81.4	10.3	2.5	153.0
Bourbon....	4 years	154.0	70.4	65.9	10.8	1.9	152.0

TABLE 93.—ANALYSES OF SCOTCH AND IRISH WHISKIES

Origin	Age	Grams per 100 liters[1]				
		Acids	Esters	Aldehydes	Furfural	Fusel oil
Scotch..........	New	25.4	61.9	11.4	6.2	199.4
Scotch..........	4 years	61.1	111.0	35.2	2.8	160.8
Scotch..........	8 years	48.0	89.7	14.2	4.0	200.0
Irish............	New	20.9	7.6	6.5	0.4	174.0
Irish............	8 years	41.8	20.9	11.2	3.4	204.0

[1] See note to Table 89, p. 560.

are used to mix with or substitute for straight whisky, are given below, the figures in each case being reduced to 100° proof for better comparison with the analyses of whisky previously quoted. Other examples are given on page 560.

The comparative ease with which the substitution of neutral spirits or inferior whisky for a high-grade straight article can be detected, provided the analysis or characteristics of the original product are known, is shown by the following illustration taken

	Grams per 100 liters of proof spirit					
	Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
1	5.1	3.2	3.9	1.2	0.0	15.0
2	...	13.3	20.4	0.5	0.0	6.3
3	1.6	2.4	32.1	5.5	0.0	19.8
4	...	2.5	3.6	0.1	0.0	2.9
5	2.4	7.2	26.4	6.0	0.0	28.0

from analyses made by the Internal Revenue Bureau preparatory to instituting legal proceedings for such a violation of the statutes (Table 94).[1]

The possibility of doing this rests, of course, on whether the increase in color, solids, and "congenerics" which results on the usual storage is sufficiently uniform to be comparable in different packages of approximately the same age. Exhaustive series of analyses made by the Internal Revenue Bureau show conclusively that this is possible, the variations in composition of the whisky from different barrels kept under the same conditions being no greater than those due to the analytical methods themselves. The individual packages affect only the depth of color (not its composition), and to a less degree the amount of aldehydes and furfural. Many analyses and a valuable discussion

TABLE 94.—COMPARISON OF GENUINE AND ADULTERATED WHISKIES

No.	Approx. age	Proof	Calculated to proof							Water-insoluble color	Color soluble in amyl alcohol	Genuine color calculated	Qual. color tests (p. 576)	Sum of congenerics
			Solids	Color	Acids	Esters	Aldehydes	Furfural	Fusel oil					
1	6 yrs.	109	190	13.8	79.3	79.9	17.0	1.7	170	79	76	10.5	Genuine	347.9
2	6 yrs.	109	191	14.0	88.0	79.9	17.0	1.8	179	74	80	11.2	Genuine	365.7
3	6 yrs.	109	202	14.5	81.5	75.5	15.6	1.8	208	76	80	11.6	Genuine	382.4
4	6 yrs.	109	199	14.7	87.0	80.4	14.7	2.0	198	75	78	11.4	Genuine	382.1
5	6 yrs.	109	136	12.8	25.3	29.7	4.2	0.6	67	9	26	3.3	Caramel heavy	126.8
6	4 yrs.	104	153	10.6	76.1	67.7	11.2	1.1	142	77	87	9.2	Genuine	298.1
7	4 yrs.	105	167	13.1	84.6	79.1	11.0	1.4	163	77	76	10.0	Genuine	337.1
8	4 yrs.	105	167	13.3	81.1	69.1	12.2	1.5	137	74	76	10.1	Genuine	298.9
9	4 yrs.	105	151	12.4	76.6	70.5	11.0	1.2	132	74	80	9.9	Genuine	291.3
10	4 yrs.	105	125	12.4	21.7	26.1	2.3	trace	51	00	17	2.1	Caramel heavy	101.1

[1] ADAMS: *Ind. Eng. Chem.*, **1911**, 647.

of the points brought out will be found in the original paper, the figures given below being typical of the differences found.

The samples suspected of not being genuine are No. 5 in the first group and No. 10 in the second. The figures show plainly the close agreement in essentials among the genuine samples kept under the same conditions of storage, and also that the chief points of resemblance between the genuine and suspected samples are in the proof and amount of color. These are, of course, easily adjusted at will to suit any conditions desired. In all other respects there is a marked discrepancy, this being especially noticeable in the acids, esters, and fusel oil, the latter being only about one-third as much in the suspected samples. The great differences in the character of the color, as shown by the proportion insoluble in water and the percentage soluble in amyl alcohol should be noted. The qualitative tests indicated the color of the genuine samples to be naturally derived from the oak wood; that of the suspected whisky was largely artificial. The sum of the congenerics was again only one-third as much in the suspected as in the genuine samples. The acids, esters, and composition of the color formed the main reliance of the chemist in prosecuting the suit.

Finally, the following analyses, taken from the records of actual cases,[1] bear out the contention of the chemist that the products in question, labeled as being "Pure Straight Whiskey" —"100 Proof Guaranteed Straight Whiskey"—"No Blend. No Compound. No Imitation" were not properly so marked.

Sample	Proof	Color insoluble in amyl alcohol, per cent	Water-insoluble color, per cent	Grams per 100 liters of proof spirit					
				Solids	Acids	Esters	Aldehydes	Furfural	Fusel oil
A	75.5	72.0	0.0	647.4	23.0	15.8	2.0	0.13	37.0
B	100.3	70.0	...	129.6	12.0	8.8	2.4	0.2	28.1

Adulteration and misbranding were alleged on the ground that the product was not a pure straight whisky but an article consisting of neutral or redistilled spirits, artificially colored in imitation of straight whisky and in one instance reduced with water

[1] Food and Drug Act, *Notices of Judgment* **343** and **3604**.

to a much lower proof than pure straight whisky. A verdict was rendered in each case favorable to the government.

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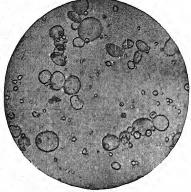
FIG. 83.—Wheat starch × 250.
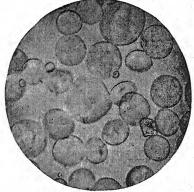
FIG. 84.—Rye starch × 250.
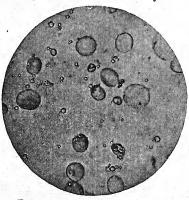
FIG. 85.—Barley starch × 250.
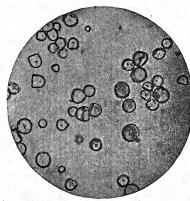
FIG. 86.—Tapioca starch × 250.
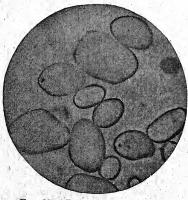
FIG. 87.—Potato starch × 250.
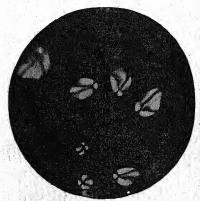
FIG. 88.—Potato starch × 200. By polarized light.
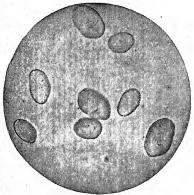
Fig. 89.—Arrowroot starch × 250.
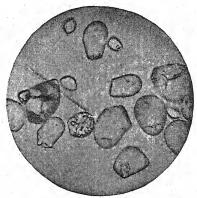
Fig. 90.—Sago starch × 250.
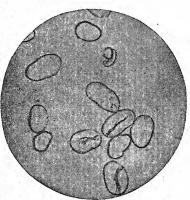
Fig. 91.—Pea starch × 250.
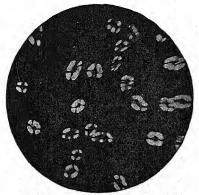
Fig. 92.—Pea starch × 200. By polarized light.
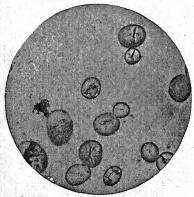
Fig. 93.—Bean starch × 250.
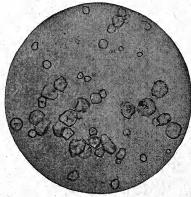
Fig. 94.—Corn starch × 250.
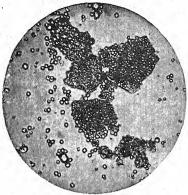
FIG. 95.—Buckwheat starch × 125.
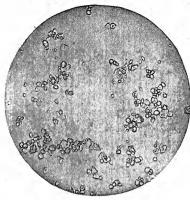
FIG. 96.—Oat starch × 250.
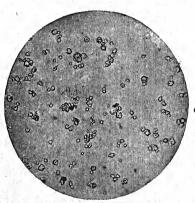
FIG. 97.—Rice starch × 250.
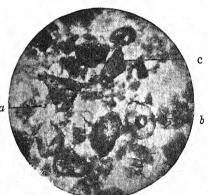
FIG. 98.—Allspice × 125. Showing starch *a*, stone cells *b*, and lumps of resin *c*.

FIG. 99.—Clove Stems × 125. Note characteristic vascular duct *a*.

FIG. 100.—Ground Coconut Shells × 125. Showing spindle-shaped stone cells and dotted trachea *a*.

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FIG. 101.—Ground Olive Stones ×
100. Showing long spindle-shaped
stone cells.
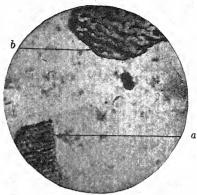
FIG. 102.—Cayenne × 35. Showing
fruit epidermis *a*, and seed epidermis *b*.

FIG. 103.—Pea Hull × 200. Trans-
verse section showing outer layer of
palisade cells.
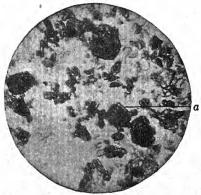
FIG. 104.—Powdered Pea Hulls ×
100. Note pea starch and groups of
palisade cells *a*.
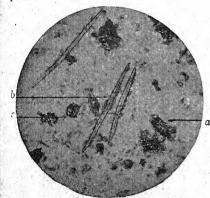
FIG. 105.—Cinnamon × 100. Show-
ing elements of powder; wood fibers
a, bast fibers *b*, and stone cells *c*.
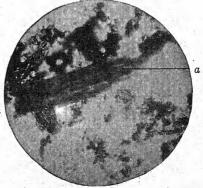
FIG. 106.—Ground Elm Bark × 100.
Note wood fibers at *a*.

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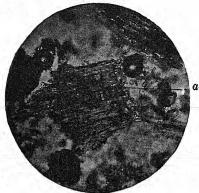
FIG. 107.—Sawdust × 100. Note spindle-shaped tracheids *a* with lateral pores.
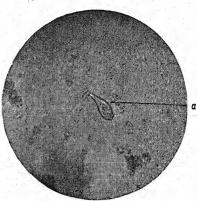
FIG. 108.—Cassia Buds × 200. Showing trichome *a*.

FIG. 109.—Cloves × 100. Showing general cellular tissue and bast fibers.
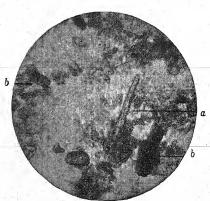
FIG. 110.—Adulterated Cloves × 100. Showing bast fibers *a* and vascular ducts *b, b* of clove stems.
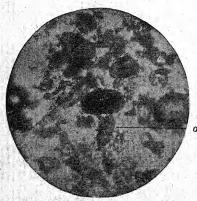
FIG. 111.—Adulterated Cloves × 100. Note the characteristic resin of allspice *a*.
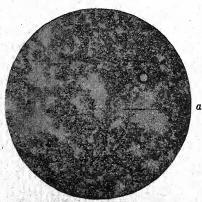
FIG. 112.—Powdered Cocoa × 100. Showing fragmentary tissues and at *a* a characteristic starch doublet.
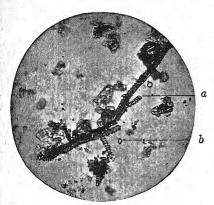
Fig. 113.—Adulterated Cocoa × 125. Showing the spiral vessels *a* and *b* of cocoa shells.
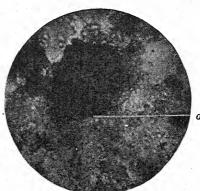
Fig. 114.—Cocoa Shells × 125. Showing the stone cells *a*.
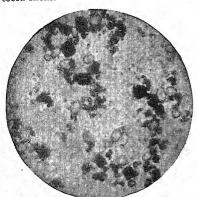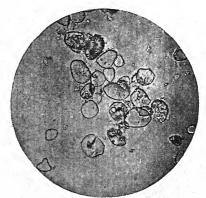
Fig. 115.—Adulterated Cocoa × 125. Showing the larger starch grains of arrowroot.

Fig. 116.—Ginger × 250. Showing chiefly the starch, characterized by a protuberance at one end.

Fig. 117.—Ginger × 125. Showing starch grains, scalariform vessels *a* and wood fibers *b*.
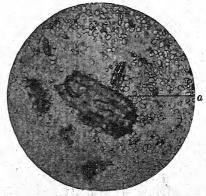
Fig. 118.—Adulterated Ginger × 100. A large fragment of the seed epidermis of cayenne is shown at *a*.
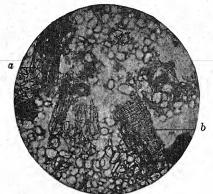
Fig. 119.—Adulterated Ginger × 125. Compare woody fibers of ginger *a* with coarser tracheids of sawdust *b*, the adulterant.
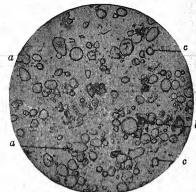
Fig. 120.—Adulterated Ginger × 125. Compare the starches, *a*, *a*, ginger; *b* wheat; *c*, *c*, corn.
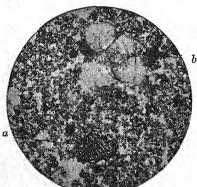
Fig. 121.—Mustard × 100. Showing mass of cellular tissue, palisade cells *a*, and colorless epidermal cells *b*.
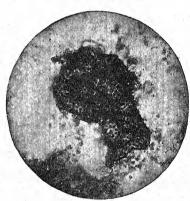
Fig. 122.—Mustard Hulls × 100. Note characteristic mass of the palisade cell layer.
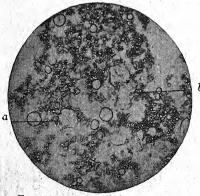
Fig. 123.—Adulterated Mustard × 125. Showing wheat starch (*a*) and the "paste-balls" and starch of turmeric *b*.
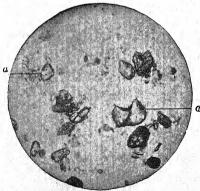
Fig. 124.—Pepper × 100. Showing characteristic aggregates of starch grains *a*, *a*.
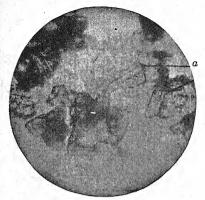
FIG. 125.—Pepper Shells × 100. Showing large proportion of stone cells a.
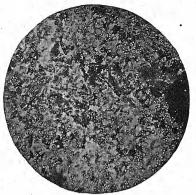
FIG. 126.—White Pepper × 200. Note starch grains and numerous crystals of piperin.
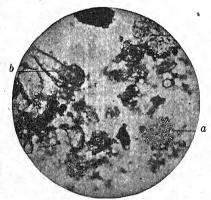
FIG. 127.—Adulterated Pepper × 125. Showing masses of pepper starch b and buckwheat starch a.
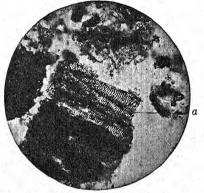
FIG. 128.—Adulterated Coffee × 100. Showing the reticulated ducts of chicory a.
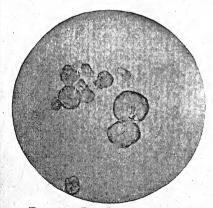
FIG. 129.—Pure Butter × 125.
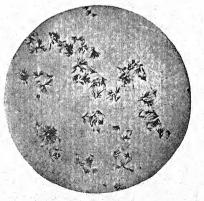
FIG. 130.—Pure Coconut Oil × 45.
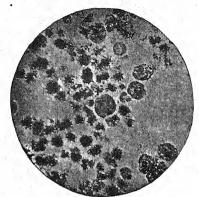
FIG. 131.—Butter with 10 per cent. of coconut oil × 125.
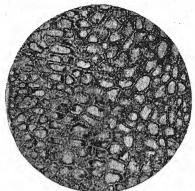
FIG. 132.—Parenchyma of Coffee × 125.

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